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REDACTORES:

E. MUSTAKALLIO (TURKU) U. UOTILA (HELSINKI) ARMAS VARTIAINEN (HELSINKI)

A. VILSKA (HELSINKI) A. I. VIRTANEN (HELSINKI)

EDITOR

K. O. RENKONEN

REDIGENDOS CURAVIT

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STUDIES ON THE DETERMINATION OF COLLOID OSMOTIC PRESSURE

WITH SPECIAL REFERENCE TO ITS CLINICAL APPLICATION

BY

LAURI AUTIO

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE WIHURI RESEARCH INSTITUTE

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PREFACE

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Helsinki, September 1954

Lauri Autio

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ON THE CONCEPT OF OSMOTIC PRESSURE

Osmotic pressure is one of the so-called colligative properties of solutions. These properties are dependent upon the number but not the nature of the solute particles. Although osmotic pressure measurements have become an important means of investigation, with applications in various fields, the concept of the essential nature of the phenomenon itself is not yet definitely established.

Probably the most widely accepted view is that based on the kinetic theory. The molecules of the solution collide ceaselessly with the walls of the containing vessel. For manifestation of the osmotic forces it is necessary that at some point a »wall» of the vessel consists of a semipermeable membrane, which permits the passage of the molecules of the solvent but not of those of the solute. The membrane thus forms for the latter a barrier similar to the other walls of the vessel. Since a further necessity is that external to the membrane there is pure solvent, it may be presumed that the solvent molecules outside the membrane collide with the membrane more frequently than those on the inside. As a result, the solvent flows through the membrane to the side where the solution is. The number of collisions against the membrane seek to attain equilibrium on the two sides of the partition (Mee 1947). — Thus osmosis is not the flow of solute molecules but of the solvent molecules (Bladergroen 1949). However, it is dependent on the ratio between the solute particles and the solvent, *i.e.*, on the concentration of the solution. The flow of solvent into the vessel causes a rise in the pressure within it (Maximov 1938).

The term osmotic pressure has been employed for slightly diverging concepts in different connections. According to Schade and Claussen (1924), osmotic pressure is a property of solutions as such, and it is measurable without the presence of an outer solvent. A more widely accepted opinion is that osmotic pressure is manifested only under certain conditions, *i.e.*, when the solution and

the solvent are separated by a semipermeable membrane. Bladergroen (1949) considered it more correct to speak of osmotic suction rather than of osmotic pressure. Every solution has an osmotic potential of a given magnitude (Levitt 1951). In his opinion, all pressure due to osmosis should be termed osmotic pressure.

The kinetic theory places no restrictions on the size of the particles in the solution. They may be ions or molecules, or also colloidal particles. The only prerequisites are that they move freely with respect to each other, are far apart in relation to their diameter, and are practically free from mutual attraction (Eucken 1944).

The osmotic pressure of colloidal solutions is considerably more complex than that of crystalloid solutions. Accurate indirect methods are available for the measurement of the latter, such as, for instance, the determination of a depressed freezing point. This method is not applicable to measurement of the osmotic pressure of colloidal solutions for two reasons. The number of colloidal particles is small in comparison to the proportion of particles in salt solutions. Calculation of the depression of the freezing point of an 1 per cent solution of colloid having a molecular weight of 20,000 will give a depression of 0.001° C, and the measurement of such minute differences in temperature is difficult. Still greater difficulty may be caused by salts which are present as »impurities». If the concentration of such salts is 0.1 per cent and the molecular weight 70, the freezing point will be depressed by 0.0027° C (Alexander and Johnson 1949).

Measurements of the osmotic pressure of colloidal solutions are made with the aid of a semipermeable membrane which is impermeable to the colloid under investigation. The principle consists in the compensation of the »osmotic suction» by a readily measurable force. This leads to the practical interpretation of the concept of osmotic pressure. The osmotic pressure of a solution is the pressure which must be applied to prevent the passage of the solvent through the membrane to the side of the solution (Bull 1951).

As used in this report, the term »colloid osmotic pressure» designates the force which is barely adequate to prevent the passage of water through the membrane to the side of the colloidal solution investigated.

For instance, the osmotic pressure of the above mentioned colloidal solution, for which the depression of the freezing point

was calculated to be 0.001° C, is equal to the hydrostatic pressure of a water column 10 cm high, which forms a readily demonstrable quantity.

The colloid osmotic pressure is a complex phenomenon and it involves factors other than the osmotic pressure which is associated with the number of colloidal particles and for which the term colloid pressure has been employed (v. Farkas 1938). A part of the measured pressure is produced by hydration of the colloid, called the swelling capacity (Quellungsdruck) (v. Farkas 1938). Even should hydration and swelling not be regarded as one and the same phenomenon, the result in any case is a decrease in the amount of »free water» in the solution. As the colloid binds water from the solution, a so-called »nichtlösender Raum» arises (v. Farkas 1938). As a result, the osmotic pressure in the higher concentrations may rise more abruptly than would seem to be called for by the increase in the colloid concentration (Staudinger 1941) (Fig. 3, p. 44).

The ions in the solution may influence the colloid either directly or through the Donnan equilibrium. If the electrolytes alter the hydration of the colloid in a manner to decrease the amount of fixed water, the tendency to aggregation of colloid particles may be facilitated. The number of particles in the solution is reduced, the degree of dispersion is changed and the osmotic pressure lowered (Haller 1929). Diverging opinions have been presented regarding the effect of the Donnan phenomenon, which is dependent upon the relationship of the colloid and electrolyte concentrations, among other factors (Hecht 1925, Wreschner 1929, Meyer 1932, Butt and Keys 1937, Alexander and Johnson 1949, Bull 1951). The theory of the Donnan phenomenon is based on the view that colloid is a colloidal electrolyte (McBain and Bolduan 1943). The effect of ions may also be manifested as so-called anomalous osmosis (Loeb 1922a, b, c, d, 1923 a, b,; Preuner and Roder 1924, Clarke 1954). Reference should also be made to the osmotic pressure of the electrolytes themselves, which is eliminated by the permeability of the membrane in the measurement of colloid osmotic pressure. Colloid osmotic pressure determinations thus yield results which are influenced by a number of factors.

The results are also dependent to some extent upon the method employed (table 1). In physicochemical investigations, measurement of the colloid osmotic pressure is used chiefly for the determination

of molecular weights. As is seen in table 2, the results obtained agree well with those obtained by the ultracentrifuge.

TABLE 1

COLLOID OSMOTIC PRESSURE OF NORMAL SERUM, IN CENTIMETERS OF WATER PRESSURE, ACCORDING TO METHOD OF SCHADE AND CLAUSSEN

28.5—36.5	Schade and Claussen 1924
28 —42	Runge and Kessler 1924
35 —40	v. Farkas 1926
30 —35.5	Marin 1927
27 —31	Fahr *et al.* 1931

COLLOID OSMOTIC PRESSURE OF NORMAL SERUM, IN CENTIMETERS OF WATER PRESSURE, ACCORDING TO METHOD I OF KROGH AND NAKAZAWA

36.5—42.5	Hecht 1925
32.5—40	Iversen and Nakazawa 1927
31.5—46.5	Ito *et al.* 1929
37.5—48.5	Oelkers 1931
29.5—40	Meyer 1931 a

TABLE 2

MOLECULAR WEIGHT DETERMINATIONS

	By Osmotic Pressure Measurements		By Ultracentrifuge	
Egg albumin	34,000	Marrack and Hewitt 1927	34,500	Svedberg 1930
Hemoglobin	68,000	Adair1925 a,b,c,	68,000	Alexander and Johnson 1949
Serum albumin ..	72,000	Burk and Greenberg 1928	68,000	—»—
Serum globulin ..	175,000	Adair and Robinson 1930, Adair and Robinson 1930	150,000	—»—
Polystyrol I	37,000	Schulz 1936	35,000	Schulz 1936
» II	90,000	» »	80,000	» »

Although the colloid osmotic pressure in biological fluids comprises only a small proportion of the osmotic pressure of electrolytes — *e.g.*, *c.* 0.5 per cent in the blood — it has a very important physiological rôle (Landis 1925, 1927, 1930, Bladergroen 1949), since biological membranes are readily permeable to electrolytes but

slowly or sparingly permit the passage of colloids. Thus electrolytes are able to become diffused more evenly in the »biological systems», whereas differences arise more readily in the colloid concentration and provide possibilities for the development of osmotic forces.

Tedious procedures are a common feature of the methods used for measurement of the colloid osmotic pressure. Probably for this reason the study of this phenomenon has been comparatively meagre in, for instance, the medical field, although Mayo wrote already in 1928: »The colloid field today presents the most promising realm in medical research.»

Measurements form the basis and criteria for the indirect methods, which strive to calculate the osmotic pressure from the colloid amounts by means of various formulas, in the effort to replace the difficult direct measurements by other, more simple analyses. For instance, the colloid osmotic pressure of the blood is calculated from the values obtained in protein analysis. Various formulas have thus been compiled, in which the colloid osmotic pressure is usually expressed as a function of albumin, globulins and total protein (Verney 1926, Govaerts 1927, Wells *et al.* 1933, Keys 1938, Goreczky and Héthelyi 1942, Bjørneboe *et al.* 1948, Meyer 1941, 1951).

Blood, however, is a complex colloidal solution and Moore and Roaf (1913) arrived at the conclusion that the colloid osmotic pressure of its main components, albumin and globulins, when measured separately and then added together is not as great as that of whole serum. They suggested that the difference is due to fluctuations in the aggregation state. It seems probable that the colloid osmotic pressure in the blood is frequently dependent upon factors other than the protein concentration to such a marked extent that the protein analysis will not give a correct picture of the condition (Reid 1904, Mayrs 1926, Verney 1926, Krogh and Nakazawa 1927, Kessler and Paulsen 1935, Zozaya 1938, Jelke 1953, Autio *et al.* 1955). As will be seen from table 3, values calculated by theoretic methods may differ greatly from those obtained by measurement. Consequently it appears that measurement is the only accurate means of determining the colloid osmotic pressure.

TABLE 3

COLLOID OSMOTIC PRESSURES OF SERUM SAMPLES CALCULATED BY FOUR DIFFERENT FORMULAS ON BASIS OF ELECTROPHORETIC PROTEIN ANALYSES, AND MEASURED VALUES FOR SAME SAMPLES, IN CENTIMETERS OF WATER PRESSURE (AUTIO *et al.* 1955). FORMULA 1: MEYER 1951, FORMULA 2: BJØRNEBOE *et al.* 1938, FORMULA 3: WELLS *et al.* 1933, FORMULA 4: GORECZKY AND HÉTHELYI 1942

Serum No.	Total Protein %	Measured Value	Calculated Value			
			Formula 1	Formula 2	Formula 3	Formula 4
1	5.6	22.5	25.5	17.5	18	17.5
2	4.8	22	25.5	22.5	20	22
3	5.6	24	19	21	21	19.5
4	6.1	26	26.5	21	22	20.5
5	6.8	36.5	33.5	19.5	22	21
6	5.9	26	20	22.5	23	21.5
7	5.6	31	30	24.5	24	23.5
8	5.8	26	28	25.5	25	23.5
9	5.7	34	29.5	26	25	23.5
10	7.0	27	33	28	29	27
11	6.6	32	32	28.5	30	28
12	6.7	35.5	35	29	30	28
13	6.9	39	33	28	30.5	28.5
14	6.7	27.5	34	29	31	29
15	7.0	36.5	35	28.5	31	29
16	6.9	30.5	35	30	32.5	30
17	7.2	40.5	35	29	32.5	30
18	7.5	34	25	28	33	31
19	7.0	38	40.5	32.5	33.5	31
20	7.1	33	26	32	34	31.5
21	6.9	30.5	35	31.5	34.5	32
22	6.9	31	36.5	31	35	31
23	8.7	41	49	29	36	33.5
24	8.0	34	42.5	32.5	37	35.5
25	7.1	33.5	25	30	37	31
26	7.6	33.5	41.5	33.5	38	35.5
27	8.3	29	43.5	30.5	37	34.5

SURVEY OF MEASUREMENT TECHNIQUES

ADVANCES UP TO 1920

The French botanist Dutrochet is recorded to have been the first to study the phenomenon of osmosis and to use, in 1826, the term osmometer for his apparatus consisting of a membranous bag attached to a tube. The phenomenon of osmosis had already been discovered in 1743 by Nollet (Pfeffer 1877). Pfeffer, who also was a botanist, published in 1877 the results of his research under the title of »Osmotische Untersuchungen». His osmometer was equipped with a semi-permeable membrane prepared by precipitating copper ferrocyanide on the walls of a porous clay vessel, which provided a strong support for the membrane. Pfeffer studied, among other substances, sugar and gum arabic solutions by immersing the vessel in water. A manometer tube was attached to the vessel, and the latter was then filled with the solution under examination, which sucked water through the walls of the vessel. The height of the column of fluid in the tube indicated the degree of osmotic pressure. The important investigations of van der Waals on the equation for the state of substances based partly on Pfeffer's results. Morse and Frazer (1898) prepared the copper ferrocyanide membrane on the vessel by means of electrolytic treatment and obtained a membrane that was sufficiently dense for the measurement of high pressures. They determined the osmotic pressure of a 91 per cent solution of glucose to be 273 atmospheres.

Starling (1896) applied the concept of osmotic force of colloids to the study of normal and pathologic human physiology. He demonstrated by animal experiments that blood proteins are able to absorb and bind water. By arranging the circulation of a protein-free salt solution through the animal's limb he was able to produce edema, which was reduced when serum was again substituted for the salt solution. He also observed that even a slightly hypertonic

sodium chloride solution (1 per cent) was resorbed from the subcutaneous tissue into the circulating blood when the tissue was perfused with a solution containing protein. The inference was made from this that the osmotic pressure of proteins, which in itself is low, is probably of greater significance in the fluid balance of the organism than that of crystalloids. Like Pfeffer, Starling used in his investigations the »static» type of osmometer. The colloidal solution under examination was placed in a tube closed with a semipermeable membrane and was allowed to suck water until prevented by the increased hydrostatic pressure in the tube. When in equilibrium, the height of the elevated fluid column in the tube was equal to the colloid osmotic pressure. The result, nevertheless, was not a fully correct one, as the water diluted the solution under investigation.

At about the same time Cohnstein (1896) and Róth (1899) also observed the power of serum colloids to bind water. Oker-Blom (1904) moistened gelatin pieces with serum and found that their weight remained unchanged or was reduced because the serum protein prevented the absorption of water by the gelatin and in some cases even caused transference of water into the serum. When the test was carried out with a protein-free solution some of the solution was absorbed by the gelatin pieces, which thus increased in weight. Lillie (1907) studied the effect of electrolytes on colloidal solutions and concluded that the osmotic pressure of these solutions was dependent upon factors similar to the water-taking power of living cells. He suggested that the osmotic pressure of cellular colloids is probably the central force in the water balance of cells.

The osmometers of Biltz (1910) and Donnan (1911) were also constructed on the static principle. To obtain a membrane that would be as thin and have as large a surface as possible, the former utilized a basket-weave network of platinum wire, on which the membrane was prepared. Donnan's osmometer consisted of a glass cylinder closed at both ends with a parchment paper membrane, which was tightly affixed to the edges of the cylinder by means of metal plates and rubber bands. Attached to the side wall of the osmometer cylinder by melting there was a glass tube into which the fluid under examination rose. The tube was connected with a mercury manometer which gave the readings. The entire apparatus was immersed in a vessel containing water.

Methods based on the static principle proved impracticable,

particularly for biological serial experiments. Days, or even weeks, were required to attain a state of equilibrium, during which the examination fluids were subject to change. Large amounts of colloid were required and the initial concentration was altered by the inflow of water into the osmometer.

Dilution of the solution under investigation was prevented by application of the principle of counterpressure which was presented by Tammann (1892) and which rendered it unnecessary to wait until sufficient hydrostatic counter pressure gradually developed. He applied an external osmometric pressure, which prevented the inflow of water into the osmometer. In the presence of a state of equilibrium, this external pressure indicated the magnitude of the osmotic pressure.

Using this osmometer, Berkeley and Hartley (1906) obtained osmotic pressure values for sugar solutions which were of the same magnitude as those secured by the static method by Morse and Frazer.

Sörensen (1919) developed for the study of protein solutions an osmometer operating on the principle of counterpressure, or compensation. A collodion bag was connected to a capillary, which in turn was connected to the pressure system and the manometer. Known pressure could thus be applied to bear on the fluid column raised in the capillary by osmosis. — Associated with the compensation principle is the so-called »dynamic» measurement, which was successfully tested by Berkeley and Hartley (1906), using sugar solutions. The purpose of counterpressure is not to attempt to gain final equilibrium, but to observe the movements of the capillary meniscus under the action of known variable pressures. Extrapolation is used to estimate the pressure which would maintain stability of the meniscus — in other words, which would compensate the osmotic pressure. — The compensation principle of Sörensen's osmometer has later been used in some way in most types of osmometer, which differ in size, construction, membrane, amount of fluid, time of measurement, and accuracy. No essential changes have been made in the basic principle of measurement. The compensation principle, which prevents the dilution of the colloid under examination, gave the initiative to the development of micromethods, which brought about a saving in the time required for performance of the osmotic pressure measurements.

METHODS USED CHIEFLY IN PHYSICOCHEMICAL INVESTIGATIONS

The theory and measurement of colloid osmotic pressure has been dealt with by Adair in several reports (1925 a, b, c, 1926, 1928, 1929). He developed and used in his experiments no less than six or more osmometers of different kinds, depending on the magnitude of the pressure applied in each case. These osmometers operated on the »static» principle. Equilibrium was allowed to set in gradually in the course of days, or even weeks, and the values were read from the height of the column of liquid in the capillary. During the long observation time, however, the colloids tended to undergo change either in aggregation or by chemical decomposition. Attempts were made to correct this drawback in »static» direct osmometry. Adair suggested using more rapid osmometers or lower temperatures to better assure chemical stability. Measurements were therefore made less time-consuming by reducing the capillary diameter and reducing the volume of liquid under investigation. At low pressure levels, however, capillarity proved to be a disturbing factor and the osmometer was sensitive to transitory changes in temperature. For this reason Adair suggested that the capillary should not be much below 1 mm. in diameter. Capillary rise corrections will then be below 2 cm, and the effect of temperature fluctuations up to $\pm$ 2° C would be rendered insignificant. With a suitable membrane, equilibrium was attained in 24 hrs., which was regarded as sufficiently rapid. However, Adair carried out the measurements at a temperature of about 0° C, which was a marked disadvantage of the procedure.

Adair (1925c) paid special attention to the possibilities of error inherent in the measurement technique. In addition to changes in the colloids, which already were mentioned above and which may make the maintenance of stable equilibrium impossible, the attainment of a true state of equilibrium may also be disturbed by other factors, which render the direct osmometric method difficult. Permeability of the membrane to colloids may permit passage of the colloid under examination and this error may not be noticed for some time since the rate of diffusion is so small that several days may pass before demonstrable amounts appear on the side of the outer fluid. This will give excessively low pressure values. Rarely the membranes

are impermeable to salts, which will produce excessively high values. Some membranes may adsorb proteins, thus reducing the concentration, while others may be too elastic and cause a change in the volume within the osmometer. The most common error arises from adhesion of the meniscus to the walls of the tube by the action of capillary forces, giving an impression of equilibrium at too early a stage. Excessively slow movement may also be misinterpreted as the final state. In the initial stages of diffusion, before the salts are in balance, there may occur transitory diffusion pressures that may be considerably greater than the true osmotic pressure.

In connection with measurements of the osmotic pressure of hemoglobin, Adair (1925c) presented criteria for differentiation of true and false pressures. The pressure must be steady. The observation time was prolonged, thus eliminating errors due to changes in proteins and permeability. The change in pressure during 3 weeks was about 1 per cent, or within the limits of experimental error, and for 9 weeks it was maintained with an accuracy of 6 per cent without protein decomposition products being demonstrable in the outer fluid. It is necessary that the osmometer has measuring facilities and that the pressure is reversible, in order that the same end result may be reached when the experiment is started at different heights of the fluid level in the manometer tube. This will eliminate transient diffusion pressures. The measurements were reported by Adair to be accurate to 1.3 cm of water.

To eliminate the error factor associated with variations in the capillary rise correction, Adair (1929) constructed osmometers in which the aqueous solution of the colloid under examination caused the movement of a colored column of toluene, which rendered the capillary rise correction smaller and less variable. Since toluene osmometers require the use of a very accurate thermostat, the temperature of 0° C used in Adair's method is suitable. Thus changes in temperature produce opposite changes in the volume of aqueous liquid and of toluene, and the instrument is practically free of temperature dependency.

Adair also published, in 1949, a method for the measurement of very low osmotic pressures. The osmometer is filled with a stable colloidal solution, which exerts an easily measurable osmotic pressure. This solution is brought into equilibrium with a buffer solution outside the membrane, making the fluid level immobile.

The external buffer solution is now replaced by an unknown colloidal solution prepared with the same buffer and having a low osmotic pressure. When equilibrium again is regained, the fluid level in the osmometer has fallen through the action of the osmotic pressure of the external colloidal solution. This will exclude the necessity of making capillary rise corrections. The modification described is considered useful in the examination of dilute colloidal solutions with a low colloid osmotic pressure and of materials of a high molecular weight (Alexander and Johnson 1949).

In the physicochemical field of investigation it was particularly important to improve the accuracy of osmometers. The more high-molecular the materials under examination, the lower the colloid osmotic pressures to be measured and the greater the effect of errors on the final result. For instance, at a molecular weight of 350,000 the osmotic pressure of an 1 per cent solution is only *c.* 0.7 cm of water.

Burk and Greenberg (1930) employed a method in which the temperature was maintained at 0° C, a large volume of liquid was needed, and several days were required to obtain equilibrium. The osmometer of Schulz (1936) also took 2—3 days to come to equilibrium. His thermostat was kept at 27° C with an accuracy of 0.05° C, the volume of experimental solution was 10 cc, and the standard deviation was reported at 4 per cent. — Since capillarity proved disturbing, especially in the measurement of low pressures, toluene was frequently used as the liquid moving in the manometer tube. Contact between the toluene and the colloidal or buffer solution is over a wide interface, usually in a bulb of the tube where capillary forces are of no importance. Osmometers of this type have been used among others, by Adair (1925c), Bourdillon (1939).

Although »dynamic» osmometry based on the rate of diffusion has been regarded as unreliable (Wells 1932, Pauli and Fent 1934) this principle also has been adapted to physicochemical investigations of colloid osmotic pressure. The best known of these methods are that of Montonna and Jilk (1941) and the modification of Fuoss and Mead (1943). A large membrane is clamped between two half-cells to separate the experimental and the outer solutions. The flow of the solution under examination is observed in a capillary tube when excess pressure is produced by varying the height of the outer fluid

level. The pressure at which no movement would occur is calculated from the rate of flow. In the modification of Fuoss and Mead the external pressure is varied, being initially above and then below the osmotic pressure. The movement of the meniscus level is observed at intervals of 30 sec. for about 6—8 min. The entire procedure requires about 1 hr. The accuracy is reported to be $\pm$ 0.05 cm of water. This method has been employed chiefly for the study of high molecular polymers (Bull 1951). A survey of methods has been presented by Weissberger (1945).

Diverging from the above mentioned methods, Jullander and Svedberg (1944) developed an osmotic balance with an accuracy reported to be ten times as great as that of other methods. An osmometer made of aluminum and glass is suspended on a balance and the diffusion of the outer fluid through the membrane into the osmometer is demonstrated as an increase in weight. Control of the experimental conditions is important in this procedure, as the temperature should not vary more than 0.005 °C during the 2—3 days required for weighing. No capillary is needed, which probably is an advantage that will promote the use of the dynamic method of determination.

METHODS APPLICABLE TO CLINICAL USE

Schade and Claussen (1924) adopted the term »oncometer» for the osmometer in desiring to differentiate the concepts of oncotic and osmotic pressure. Thus the latter term would designate only the »true» osmotic pressure produced by the number of colloid particles. Oncotic pressure would be that actually measured in the colloidal solution; in other words, the complex of phenomena with which is associated not only the »true» osmotic pressure but also the swelling of colloids and the pressure produced by the unequal distribution of ions. Although the term oncotic pressure has also been used later, its justification has been contested, since measurement of the oncotic pressure would be oncometry — a term which has a connotation entirely different from the investigation of colloids (Meyer 1932).

The method of Schade and Claussen is based on the idea that the osmotic pressure of a colloidal solution »exists» in the solution and may be measured without the aid of an external liquid. The solution

under examination is pressed from below against the membrane, which is covered with a layer of mercury. An ultrafiltrate passes through the membrane and is pushed off the membrane by the mercury. The object is to find the pressure at which no filtrate passes through the membrane and the fluid level in the capillary of the osmometer remains stable. By using excess pressures of various magnitudes and observing the rate of filtration at each pressure, the pressure at which no filtration would occur is obtained by extrapolation. The osmometer chamber which contains the fluid under examination is connected by means of a capillary to a water manometer and two water flasks. When the relative heights of the fluid levels in the two flasks is varied, excess pressures registered by the manometer are transmitted through the capillary into the osmometer. The rate of movement of the fluid level in the capillary, which manometer are transmitted through the capillary into the osmometer. The rate of movement of the fluid level in the capillary, which is observed through a horizontal microscope, is an indication of the rate of filtration. The pressure against the membrane produced by the mercury must be taken into consideration. In the opinion of Meyer (1932), Schade and Claussen were in error regarding their method in the respect that ultrafiltrate is deposited on the membrane and forms a thin layer beneath the mercury; it is with this filtrate that the liquid establishes equilibrium. About 3 hrs. are required for measurement by this procedure. Its disadvantages are the use of a thermostat at 37° C, preparation of the membrane on a supporting network, and the large amount of solution needed, 20 cc of blood being required for a parallel determination of the colloid osmotic pressure of plasma. The standard deviation was stated to be $\pm$ 2.5 cm of water.

For improvement of this method, Kylin and v. Pein (1931) developed a modification based on the same principle but involving certain practical alterations. In their opinion the most important points were the following: Measurement should be made against the ultrafiltrate of the experimental solution or against Ringer's solution; working should be possible at room temperature, the membrane should be easily attachable, the time of experiment should be shortened and reliability improved. The specially made membrane was replaced by one obtainable ready made, the thermostat was eliminated, and filling of the osmometer was simplified.

Since a given amount is filtered at a given pressure, the meniscus in the capillary moved at a known rate, and the number of divisions which it moved per minute was determined through an ocular micrometer. Three pressure values were selected and the colloid osmotic pressures corresponding to the rates of movement were found on an empirically compiled curve. The readings were obtained 10 min. after the application of pressure and the entire measurement procedure took less than an hour. Parallel determinations gave results showing a difference of not more than 3 cm of water pressure.

The osmometer of Govaerts (1924 a) consisted of a metal disc 25 mm in diameter and 10 mm in thickness, which comprised the osmometer chamber. Its upper part was tapered to enable connection with the capillary tube, and the lower aperture, to which the membrane was affixed, was large. For the latter, commercial cellophane was clamped between two metal rings. The chamber was filled with the liquid under examination, which rose into the capillary. Against the undersurface of the membrane was placed the outer colloid-free liquid, contained in its own vessel. Because of the free surface of the outer liquid the osmometer could not be placed in the water thermostat but was kept at room temperature. To prevent evaporation of the outer liquid the apparatus was placed in a vessel with water on the bottom. It was considered that equilibrium was established in 24 hrs. The capillary tube was closed with a cock, which now was opened and the capillary connected to the manometer. The capillary meniscus was observed with a horizontal microscope for determination of the pressure at which it would remain stable. The colloid osmotic pressure was the sum of the manometric pressure and the height of the liquid column in the capillary tube when in the state of equilibrium, with deduction for the effect of capillarity. Parallel tests showed a deviation of $\pm$ 1—1.5 cm of water pressure provided the room temperature was constant. Disadvantages of this method were the length of time required and the sensitivity to changes in temperature.

To correct the latter drawback, Verney (1926) altered the construction of the osmometer by making it immersible in water, which could be maintained at a more constant temperature than the room. He inverted the apparatus, so that the outer fluid lay freely on the membrane, placed the osmometer into a flask, bent the capillary to form two right angles, and connected a number of

osmometers to a common manometer, enabling the simultaneous performance of several measurements.

The best known and probably the most widely used osmometers of this kind are models I and II published by Krogh and Nakazawa (1927). The osmometer used in method I resembles in principle Sörensen's apparatus in miniature. Measurements are made according to the method of Govaerts. By submerging a capillary tube several times into the collodion solution a shell of collodion is cast around the tube. This shell is removed and forms the membrane. Controls are made of its water-permeability and protein-tightness. It is filled with the colloidal solution under examination, of which it can take only 0.4 cc, and is fastened with a rubber band to the lower end of a capillary tube. It is then placed in a small test tube, which barely accommodates the collodion shell and into which the outer liquid is poured. The capillary, collodion shell and small outer tube containing the external fluid are placed with the aid of a rubber cork into a larger test tube with mercury on the bottom. This raises the small test tube with the outer fluid near the membrane. The entire apparatus is placed in the water bath. The membrane offers a fairly large diffusion surface of *c.* 3—4 cm^2. As, furthermore, the amounts of liquid used are very small, *i.e.*, 0.4 cc of osmometer fluid and *c.* 0.1 cc of outer fluid, equilibrium develops between the fluid under examination and the diffusible particles in 4—5hrs. In the case of measurement by the Govaerts's method, Krogh recommended a control measurement 16—24 hrs. later but according to Meyer (1932) this is not necessary. The accuracy of *c.* 1 cm of water reported by Krogh is in Meyer's opinion (1932) a very cautious estimate.

Meyer (1932) made several investigations with the osmometers of Krogh and Verney, placing into the same thermostat 12—24 osmometers which were connected to a common manometer. In addition, two side tubes were affixed to each osmometer chamber, so that a gaseous mixture could be conducted through the osmometer to study its effect on the colloid osmotic pressure. Meyer reported that he had carried out nearly 3,000 parallel determinations, with a maximum deviation of only $\pm$ 0.2 cm of water.

As the preparation of the membranes was considered tedious, especially for clinical use, ready-made collodion, cellophane or fish bladder membranes were employed in Krogh's method II. The

apparatus resembled Govaerts's osmometer but was not constructed of metal but of hard rubber. For the outer fluid was substituted a piece of filter paper moistened with Ringer's solution and placed against the undersurface of the membrane. Below the filter paper was a small rubber funnel, within which there was a small bubble of air when the osmometer was immersed in the water thermostat. The bubble prevented contact between the water in the thermostat and the outer fluid. Since the volume of outer fluid was small, excess pressures alone are usable, as in the method of Schade and Claussen. In reading the measurements, consideration should also be given to the depth at which the osmometer lies in the thermostat. The reported deviation in this method is $\pm$ 0.75 cm of water.

Del Baere (1931) suggested placing a glass rod into the collodion bag in order to reduce the space available to the solution under examination, so that the fluid would, in a way, be »interface only». Establishment of equilibrium would then require only ½—1½ hrs. It has been assumed, however, that such small amounts of fluid may be subject to error because of dilution of the experimental solution by osmosis (v. Farkas 1938). Meyer (1932) has also expressed doubts concerning the suggested improvement, stating that Del Baere had obtained osmotic pressure values that greatly differed from those of other investigators.

There are numerous modifications of the methods of Krogh and Nakazawa. Turner (1932) studied the influence of various membranes on the results and observed that concordant pressure values were obtained provided the membranes were impermeable to proteins. It thus appeared improbable that the electrical properties of the membranes would exert any influence. He placed six osmometers on the same rack, connecting them to a common manometer, increased the pressure gradually, and 3 hrs. later either increased or lowered the pressure in the manometer, depending on the direction in which the meniscus was moving. Equilibrium was established and readings were obtained in 6—20 hrs. Equilibrium could be maintained for over 30 hrs.

Fellows (1932) found a cellophane membrane best suited for his modification. He particularly stressed the importance of asepsis and of an accurately controlled temperature, to which Govaerts, Krogh and Nakazawa, and Turner had given little consideration. Fellows obtained with his osmometer lower values than many other workers.

Hill's (1933) modification required only 0.3 cc of solution and the measurement time was reduced by one-third.

Pauli and Fent (1934) performed careful investigations of the colloid osmotic pressure of serum albumin with an apparatus modified from Krogh's osmometer, in which special attention was paid to accuracy of measurement. They reported that every third measurement on Krogh's osmometer was unsatisfactory and that negative pressure values even were obtained. This was ascribed to small leakages which were claimed to occur readily at certain points, for instance at the edges of the membrane. The observation time was extended, temperature control was improved, and to prevent changes in the proteins the determinations were carried out at a temperature of + 1.5 °C. Control analyses were made of the solution in the osmometer for possible errors in dilution. Accuracy was improved at the expense of facility, and the measurements were rendered too tedious for use in serial examinations.

Keys and Taylor (1935) took into use again the static type of osmometer. The compensation principle was not utilized and the osmometer consisted only of the chamber with membrane and the capillary tube to show elevation of the fluid. The authors considered as disadvantages of other methods the complicated apparatus and the special training required for their utilisation. The use of an ultrafiltrate as external liquid was also unnecessary in their opinion, agreement not having been reached on this point at the time. It was pointed out that in nature, as a rule, there is no blood ultrafiltrate external to the capillary wall. In the method of Keys and Taylor, the serum is diluted with an amount of buffer solution equal to that used as external liquid. The filled osmometer is placed in the ice box or refrigerator room at a temperature of 0° —+5° C ± 2° C. The readings are obtained from the height of the meniscus level 3, 4 and 5 days later. According to the authors the advantages of the method are the identical pH values and salt concentrations on both sides of the membrane from the outset of the experiment. Owing to the low temperature, sterility during filling was not necessary regardless of the lengthy diffusion time. In parallel measurements by their own method and that of Krogh and Nakazawa, Keys and Taylor obtained lower values by the former, with deviations of 2—5 cm of water. They ascribed these variations partly to differences in pH and salt concentration. Despite the simplicity of

the apparatus, the length of time and the low temperature rendered this procedure unserviceable for clinical use.

There was still a lack of methods suitable for clinical use, for differences up to 20 per cent were obtained in parallel determinations by the methods of Schade and Claussen and of Kylin and v. Pein, while the method of Krogh and Nakazawa was too slow (Hepp 1936). A great improvement in both respects was provided by the procedure developed by Hepp (1936), in which the measuring time was 30—35 min. and the accuracy $\pm$ 2 mm of water. The osmometer capillary, in which movement of the meniscus is observed, is situated on the other side of the membrane, *i.e.*, on the side of the external liquid. The liquid under examination, of which there is *c.* 0.4 cc, forms a layer of *c.* 1 mm on the membrane. The capillary is in a horizontal position and the height of the capillary fluid level need not be considered in the calculations. Several consecutive measurements can easily be made, as only about 5 min. are required for the cleaning and re-filling of the osmometer. The principle of operation is the dynamic compensation measurement and observation of the rate of filtration of the liquid at known pressures.

Peters and Saslow (1940) performed a critical study of the serviceability of Hepp's osmometer and reached a favorable conclusion concerning accuracy and speed of operation. They made measurements of the colloid osmotic pressure of, for instance, horse serum albumin and on the basis of the results obtained calculated the molecular weight to be 73,400. Ultracentrifuge determinations have given a weight of 72,000 $\pm$ 3,000, according to Burk (1931). They also examined various membrane materials and found the permeability of cellophane membranes too limited and uneven. In experiments using cellophane membranes on Krogh's osmometer, the colloid osmotic pressure of serum samples occasionally did not change although the samples were diluted up to 50 per cent and parallel determinations were made in all cases. Giges and Kunkel (1954), using Hepp's method, have reported a deviation of 7 mm of water.

Since Hepp's osmometer was not very easily constructed, Rehm (1944) developed a simplified model of the apparatus. In this method, changes of 2 cm of water pressure are made from time to time, causing reversal of the movement of the meniscus. Rehm

employed cellophane membranes after determining that their permeability is less variable than that of collodion membranes.

A micromethod based on static compensation measurements for use in clinical and physiological research work has been published by Holm-Jensen (1949). The flow of water into the osmometer is compensated by counterpressure, and final equilibrium is attained in 1 hr. by the use of small volumes of liquid and magnetic stirring. The volume of experimental fluid is *c.* 0.5 cc and the accuracy *c.* ± 1 cm of water pressure. This osmometer will be described in detail in the following chapter on the technique employed in the present investigation. This method of pressure measurements is very suitable for physiological laboratory investigation but it is too complicated for use in clinical laboratories.

Still smaller volumes of solution are needed in Christensen's method (1950), which requires only 0.05—0.1 cc of fluid. The deviation from the mean is reported to be ± 06.—0.3 cm of water pressure. The moving meniscus in the capillary is observed during 3 min. at four different pressures by means of a cathetometer provided with a micrometer. The pressures are 2 cm and 4 cm above and below the pressure at which the meniscus is approximately stationary. However, these measurements can be made only after the osmometer has been filled for 24 hrs. with the liquid under examination. The colloid osmotic pressure value corresponding to the rate of movement is obtained from an empirically calculated curve. This procedure is accordingly based on the known principle of dynamic compensation and its disadvantage is the long time required for measurement.

The static osmometer principle was used once more as the basis of the method presented by Campbell and Haddy (1950). The membrane is clamped between metal rings, and attached to the osmometer there is a capillary tube into which the colloid rises. Into the same tube, over the colloid, is placed a column of mercury. The position of the top surface of this column is read from the scale on the tube. It is reported that equilibrium is established in 8—14 hrs., the height of the column of mercury then designating the colloid osmotic pressure after the capillary rise correction is made. The standard deviation is stated to be ± 1 mm of mercury.

RECENT METHODS USING ELECTRIC MANOMETERS

A great saving in time is reported to have been effected by the use of electric pressure measurements.

Hansen (1952) stated that the measurement of colloid osmotic pressure is possible in 3 min. by his method. Whereas optic magnification is employed in other methods for observation of the movement or stability of the liquid, Hansen uses an electric pressure meter, which indicates the direction of flow of the fluid. The function of the electromanometer is to indicate whether or not the state of equilibrium has been attained, *i.e.*, it serves as a zero indicator. The manometer is connected to the side of the outer fluid. When equilibrium has not yet been established, the pressure in the meter will vary according to the rise or fall of the capillary meniscus, thus indicating whether the controlled external pressure on the side of the colloid should be elevated or lowered. The membrane is of collodion, with a thickness of 5—10 microns. Hansen reported that a reliable state of equilibrium can be attained in 3 min. In his opinion *the equilibrium is established in very thin layers of fluid adjacent to the membrane.* When distilled water was used as outer fluid and the osmometer contained physiological saline solution, the latter was found to have no osmotic effect. This indicated that *the diffusion equilibrium of crystalloids was very rapidly established.* When concentrated saline solution was employed there was an osmotic difference, which disappeared very rapidly. These observations seem to bear out the opinion that salts do not disturb the establishment of equilibrium even after some minutes. The maximum deviation from the mean in this method is stated to be ± 0.5 cm of water pressure. It probably has the usual disadvantages of electric pressure measurements in general, *i.e.*, the electromanometer is a fairly costly piece of equipment and the appropriate osmometer is unavoidably rather complicated in structure.

The method has been controlled by carrying out colloid osmotic pressure measurements with a 5 per cent solution of albumin and a 2.5 per cent dilution of this solution. The molecular weight of albumin was determined to be 67 000.

A still more rapid method of measurement was published by Pappenheimer and Lin (1953), who also used the electromanometer. In addition to the manometer, two small apparatuses similar to

Hepp's osmometer are required. The outer fluids below the membrane are connected to each side of a differential conductance manometer. The membrane has a support. A high pressure is applied to the solution on the top of each membrane, causing filtration into the two halves of the conductance manometer. If the solutions and membranes in the two osmometers are identical, then no differential pressure will arise during the filtration process. However, if a dissimilar solution is placed in one of the osmometers and this is restricted in its passage through the membrane, the conductance manometer will show a differential (osmotic) pressure. The authors reported that equilibrium was 95 per cent complete within 1 min.

In the methods of colloid osmotic pressure measurement described above, the static, dynamic and compensation principles are employed in various ways. The movements of the fluids are observed and registered by optical or electrical means.

INTRODUCTION TO THE SUBJECT OF INVESTIGATION

From the above review it was seen that several methods that give reliable results have been developed for the measurement of colloid osmotic pressure. In this respect there probably is very little room for improvement provided the worker masters well the method which is being employed. However, incidental factors may cause marked errors in measurement, as was observed by Pauli and Fent (1934) and Hepp (1936). Complications of various kinds may, of course, happen to the inexperienced worker more readily than to one well familiar with a given method. Logically, the more complicated the method, the more numerous the inherent possibilities of error.

With the exception of certain »static» apparatuses, a common feature of the osmometers described above was their complex structure. Detailed descriptions of the sources of error have generally been omitted from the reports, but the disadvantages and weaknesses of the methods become manifest when they are applied to use. In the case of osmometers of simple construction, again, their greatest disadvantage is the length of time required to establish equilibrium. It would appear that these two reasons — complicated apparatus and time-consuming technique — have been the chief factors limiting clinical measurement of colloid osmotic pressures. However the need of such measurements for the elucidation of numerous phenomena of pathological physiology is obvious (Altschule 1949).

When the carrying out of such measurements was found necessary in connection with a study of questions relating to congestive heart failure started in the Wihuri Research Institute in 1950, it was decided to attempt to apply the method published by Holm-Jensen (1949). This method appeared to offer possibilities for performing several simultaneous microdeterminations with sufficient accuracy and the least loss of time.

THE HOLM-JENSEN METHOD

The osmometer as devised by Holm-Jensen (1949) consists of a capillary glass tube, 18 cm long, the lower end of which widens out into a bulb, forming the osmometer chamber. The fluid under examination is pipetted into the bulb through a small side tube, which is then closed with a rubber cap. The bottom of the chamber, which is 7 mm in diameter, is a membrane of nitrocellulose, which is cast directly into the aperture. The required amount of fluid to be examined is *c.* 0.3 cc. The colloid-free outer fluid is physiological saline solution, one drop of which is placed on a disk of filter paper on the undersurface of the membrane. The capillary tube passes through a rubber stopper, with the aid of which the osmometer chamber at the lower end of the tube is enclosed in a small cylindrical glass vessel, the »moist chamber», which protects the osmometer while in the water bath. To provide atmospheric pressure, a capillary also leads out of the moist chamber.

Compared with the osmometer of, for instance, Krogh and Nakazawa, which required a colloid volume of the same order of magnitude, the Holm-Jensen apparatus has a membrane of considerably smaller area. This circumstance tends to retard the establishment of equilibrium. To remove this disadvantage, the osmometer chamber contains a small magnetic stirrer, *i.e.*, a piece of iron in a glass tube, the upper end of which is held against the wall of the osmometer by the action of a permanent magnet in the rubber stopper. In the water bath, near each osmometer, is an electromagnet which produces an oscillating magnetic field. The oscillations in the field move the stirrer, the lower end of which swings freely and puts the colloid solution above the membrane into motion. This arrangement shortens the time required to establish equilibrium between the diffusible particles. The stirrer requires a large number of additional parts. In addition to the electromagnets, which must be water tight in order to retain activity in the water bath, a blocking oscillator is required to feed the magnets with intermittent current.

The original model of the Holm-Jensen arrangement comprised four osmometers on a semicircular rack in the water bath, in which case one 500 ohm electromagnet was sufficient. On a vertical shaft in the center of the semicircle there was a horizontal reading micro-

scope for observation of the menisci in the capillary tubes. In the apparatus constructed at our laboratory, seven osmometers were placed side by side on a straight rack and the microscope was moved along two slide rods before the rack. It was therefore necessary to provide each osmometer with a separate electromagnet. The seven electromagnets were placed in one plastic trough for immersion in the water bath.

Since the pressure determinations are carried out on the principle of compensation, the upper end of each osmometer capillary is connected by means of a rubber tube to a pressure bottle, to which a manometer tube is also attached. The Holm-Jensen water bath was regulated to maintain the temperature during measurements at 25 °C, accurate to 0.01 °C.

In view of the small amounts of fluid employed, it is necessary to apply excess pressures to prevent the flow of outer fluid into the osmometer. Too great a pressure, however, may cause concentration of the colloidal solution under examination because of the filtration process. Measurements can begin *c.* ½ hr. after the osmometer, filled with the experimental solution, is placed in the water bath, the excess pressure adjusted and agitation started. The state of equilibrium is generally attained within the following ½ hr. The colloid osmotic pressure is the height of the column of water in the manometer plus the height of the column of fluid in the osmometer minus 1.2, which is the capillary rise correction for this apparatus. At this time the capillary meniscus shall have attained stability and begin to fall when the hydrostatic manometer pressure is raised by 0.2—0.3 cm, and begin to rise when the manometer pressure is lowered by 0.2—0.3 cm.

Holm-Jensen reported that parallel measurements will agree with a maximum deviation from the mean of ± 0.3 to ± 0.5 cm of water pressure. The weakest point of the method he considered to be the rubber cap applied for closing the side tube of the osmometer, which, after being placed on the tube, may change shape and consequently alter the volume of the osmometer chamber, causing corresponding movement of the capillary meniscus. Variations in the volume of outer fluid were mentioned as another possible cause of error. Especially if this volume is not adequate, the capillary suction power of the disk of filter paper will influence the measure-

ments. This danger does not exist if there is sufficient fluid on the paper so that a small hanging drop adheres to it.

Owing to the arrangements connected with the agitators in particular, the Holm-Jensen method appeared too complicated for clinical purposes. A possibility was therefore taken under consideration of combining the simple construction of static osmometers and the advantages of micro-osmometers, *i.e.*, a small fluid volume and a short measuring time.

Since some time passes before a state of osmotic equilibrium is established, the rate of diffusion is the quantity observed in dynamic determinations. However, since the reliability of methods based on the rate of diffusion has been open to doubt (Wells 1932, Pauli and Fent 1934), the *direction of flow* appeared to be a more accurate object of observation.

Proceeding from this basis, an effort has been made in the author's investigations to find *solutions to the following questions:*

1. Is it possible to measure the colloid osmotic pressure by observation of the direction of flow of a liquid when various colloidal solutions having known colloid osmotic pressures are placed on one side of the membrane? Is it possible to construct an osmometer of simple structure to which this principle may be applied?

2. If such a method is employed, do the results correspond to the values obtained with the Holm-Jensen method for (a) albumin solutions, (b) dextran solutions, (c) normal sera, and (d) various pathologic sera?

3. Are the results influenced by (a) the electrolyte concentration, (b) the hydrogen ion concentration and (c) temperature of the solution under examination?

4. What are the possible advantages of such a method as compared with earlier methods, and what are its disadvantages, especially with a view to clinical application?

AUTHOR'S METHOD OF COLLOID OSMOTIC PRESSURE MEASUREMENT

PRINCIPLE

A known colloid osmotic pressure is applied on one side of the membrane to function as the counterpressure for the colloid osmotic pressure under examination on the other side. It is then ascertained whether the flow of water through the membrane occurs towards the side of the known colloid or of that under examination. The colloid possessing the higher osmotic pressure sucks water to its own side of the membrane. When several solutions of varying concentrations and of known colloid osmotic pressures are available, it is possible to place an unknown solution between two known solutions, one of which has a higher and the other a lower osmotic pressure than the solution under examination. — The direction of flow of the liquid is also influenced by the hydrostatic pressure associated with the height of the column of liquid in the vertical capillary tube and the capillary rise, both of which are known quantities and are to be considered in calculating of the colloid osmotic pressure from the values obtained.

Methods of this type, based on the comparison of known and unknown colloids, presumably have not earlier been used, possibly because of a lack of suitable »standard colloids» whose osmotic pressure is known. Stable colloidal solutions are now available for infusion as substitutes for blood (Council on Pharmacy and Chemistry 1951).

It may be mentioned that Blegen and Rehberg (1938) employed, chiefly for the measurement of the osmotic pressure of electrolytes, a method of observing the rate of initial flow at varying concentrations of the outer fluid. Differences in the osmotic pressure were determined from the rates of flow of the liquid. The membrane was a collodion sac. Usually membranes of this type readily permit the passage of salts, leading to a rapid equalization of the difference in concentration on the two sides of the membrane.

Blegen and Rehberg were able to lower the permeability of the membranes by extending the drying period, thus greatly retarding the passage of salts.

The collodion sac was attached to a short glass tube, which was connected to a capillary tube by a rubber tube. The sac was filled with a known salt solution and placed in another known salt solution. The time required for the capillary meniscus to move a known distance on a graduated scale is observed through a microscope. The osmometer is then placed in the solution under examination and the velocity of the meniscus is again observed. The test must again be repeated with a known salt solution, as the permeability of the membrane tends continuously to become reduced. In comparisons of this method and the accurate vapor pressure determinations of Hill (1930), measurements of sera gave results that were in good agreement, the standard deviation in 58 measurements being 0.4 per cent.

The method of Blegen and Rehberg as well as the observations of Hansen (1952) may be taken as an indication that electrolytes rapidly penetrate a collodion membrane. This is an important requirement for the serviceability of the method for comparison of colloid osmotic pressures proposed above. The disturbing effect of transient »diffusion pressures» which are unconnected with the osmotic pressure of colloids need probably be given little consideration, especially if care is taken to select as equal electrolyte concentrations as possible for both sides of the membrane.

EQUIPMENT

The equipment used in this investigation comprised the following parts:

4—8 osmometers,
Supporting arm to hold the osmometers in the water bath,
Microscope, magnification *c.* 10 ×, mounted horizontally and provided with a micrometer, for observation of the capillary meniscus,
Aquarium vessel, volume 30—40 liters, for water bath,
Capillary pipettes for filling of osmometers,
Pressure bottle for testing of membranes, connected by a polythene tube to the osmometer capillary tube and provided with a graduated manometer tube for pressure readings,
Collodion solution for preparation of membranes,
Macrodex for preparation of standard colloidal solutions.

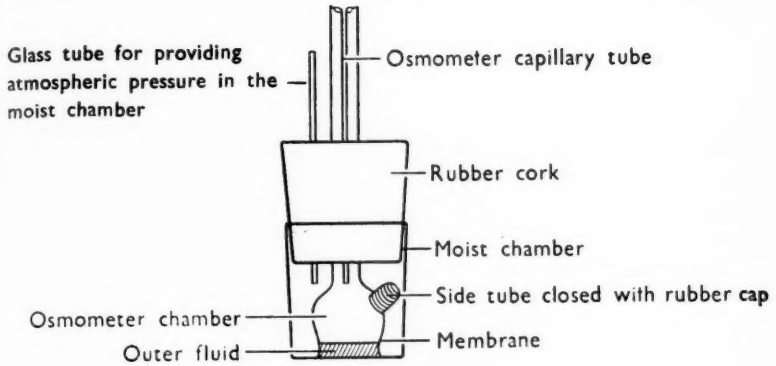

Fig. 1. — Osmometer.

OSMOMETER

The osmometer devised by Holm-Jensen (1949) was used in these experiments (Fig. 1). It consists of a capillary tube, length 18 cm and internal diameter 0.7 mm. The lower end of the tube widens out to form a chamber with an open bottom wall. This aperture is closed with a membrane 7 mm in diameter, through which osmosis takes place. For filling, the chamber is provided with a side tube, length *c.* 5 mm, internal diameter *c.* 1.5 mm. It is closed with a tight rubber cap, for instance a cystoscope stopper.

With the aid of a rubber stopper the osmometer chamber with membrane is lowered in the manner shown in fig. 1 into a cylindrical glass vessel, the »moist chamber», until the membrane lies 1—2 mm above the bottom of the moist chamber. A drop of »outer fluid» placed on the membrane is thus firmly compressed between the membrane and the bottom. For equalization of the pressure in the moist chamber, a fine glass tube passes out through the stopper.

MEMBRANE

Fick (1885) was the first to substitute nitrocellulose membranes for animal and vegetable membranes in the study of diffusion phenomena. Nitrocellulose is the general name of various nitration products of cellulose. It is prepared by treating cotton wool with a mixture of concentrated nitric and sulphuric acids, the degree of nitration being dependent on the manufacturing process. Collodion is a solution of nitrocellulose in which a mixture of ether and ethyl alcohol is used as solvent.

The most important practical problem connected with the preparation and use of nitrocellulose membranes is the question of permeability. It may be influenced by a number of factors. The effect on the permeability of the membrane of the type of nitrocellulose used was observed by Bigelow and Gemberling (1906). The kind of solvent used was found significant by Malfitano (1908), and if the solvent is a mixture of various substances the ratio of the components is to be taken into consideration (Eggerth 1921, Nelson and Morgan 1923). Maximum permeability was secured by Malfitano by using a collodion containing a large amount of alcohol, whereas Eggerth varied the permeability by changing the ratio of alcohol and ether. Nelson and Morgan employed mixtures of alcohol and water after observing the marked significance of water. The greater the amount of water remaining in the collodion after the drying process, the greater not only the permeability but also the fragility of the membrane.

According to Walpole (1915), the length of the drying period and the permeability of the membrane are inversely proportional. For permeability he employed the term »wetness», signifying the relationship between water and nitrocellulose in the finished membrane. The manifold swelling phenomena connected with the production of membranes have been studied, among others, by Brown (1915).

Important progress was made in the investigations of Pierce (1927), who demonstrated that permeability may be altered as required by the addition to the collodion of varying amounts of ethylene glycol, a hygroscopic, non-volatile swelling agent. The volatile solvents are allowed to evaporate in a dry atmosphere and the membrane gels thus produced are immersed in water. The greatest inconvenience proved to be the dry atmosphere required for the drying process. When the membranes are dried in the refrigerator (Holm-Jensen 1949) the amount of water left in them will be fairly constant owing to the low temperature and low humidity, and it will remain so for a long time because of the slow rate of evaporation of ethylene glycol.

The principle of the production of collodion membranes is thus the dissolving of dry nitrocellulose in an ether-alcohol mixture to which ethylene glycol has been added. The alcohol causes a swelling process in the nitrocellulose. The drying of the membrane is the evaporation of the ether and alcohol, the sol thus becoming gel. The course of this process is dependent upon the amount of water which will remain in the membrane and which is greatly influenced by ethylene glycol. The uniform structure, even thickness and permeability of the membrane depend upon the structure of the gel at the time the membrane is soaked in water at the end of the preparation process. In the beginning of the report of his investigation, Pierce stated as follows: »Many investigators who have attempted to use nitrocellulose membranes in their work have found the preparation of the membranes problem in itself, often more time-consuming than the projected investigation.»

Preparation of the Membrane

The membranes were produced and tested according to the method of Holm-Jensen (1949). The collodion solution was prepared as follows: Dried nitrocellulose (»Collodion Gurr», Edward Gurr, Ltd., London) was dissolved in alcohol-ether to which ethylene glycol had been added (Collodion Gurr, 4.0 g; ethylene glycol, 5.5 cc; absolute ethyl alcohol, 125 cc; ether, 80 cc). The solution can be stored in a well stoppered dark bottle for at least two years. The membrane will adhere to the osmometer more firmly if the rim of the osmometer aperture is brushed with a 10 per cent alcoholic solution of shellac, which is allowed to dry before the membrane is cast.

A small amount of collodion is poured into, for instance, the glass vessel which is to be employed for the »moist chamber» until there is a layer of *c.* 1 cm on the bottom. The aperture of the osmometer is carefully lowered so that it touches the surface of the collodion solution, which immediately forms a membrane over the aperture. The osmometer is rotated around its longitudinal axis for *c.* 10 sec., holding the membrane downwards at an angle of *c.* 10° from the vertical. It is then suspended, with the membrane downwards, on its storage rack to dry for 2 or 3 min. The membrane is then once again allowed to touch the surface of the collodion solution and the osmometer is rotated to spread the layer of collodion evenly over the membrane. Rotating is continued for 1.5—2 min., and the membrane is allowed to dry for *c.* 5 min. The osmometer is then dipped into the collodion solution to a depth of 0.3—0.5 cm, an excess drop is allowed to fall off, and the osmometer is rotated once more around its longitudinal axis at an angle of *c.* 45° for 1—2 min., to form a uniform third layer of collodion on the membrane. If the membrane does not withstand this immersion but is pressed inwards, the preceding drying phase was too short and must be extended to 10 min., for instance. When the membrane has been allowed to dry after the third dipping for 5—10 min., the dipping operation is repeated, the excess drop allowed to fall off, and the osmometer is rotated first in a horizontal position for *c.* 10 sec. and then with the membrane upwards at an angle of *c.* 45° for 1.5—2 min. When also the fourth layer is dry, the osmometer is dried in the refrigerator, with the membrane upwards, for at least 12 hrs.

A longer period of storage will not damage the membrane. After removal from the refrigerator, the osmometer is filled with distilled water and stored with also the outer surface of the membrane touching a water surface. After 2 or 3 hrs. the membrane is ready for testing.

Testing of the Membrane

Before a new membrane is taken into use, it is necessary to test its tightness by determining its permeability to water. Although the membranes are prepared in a similar manner from the same batch of collodion solution, it does not always follow that they are identical in regard to permeability. Ethylene glycol is highly hygroscopic, and variations in, for instance, the humidity of the air in the laboratory and in the drying time (Krogh 1922) may influence the properties of the membranes.

The permeability of the membrane is indicated by the so-called »minute number», which denotes the amount of water filtered through a given area of membrane during a given time under a given pressure. For determination of this minute number the osmometer is filled with distilled water and connected to the pressure bottle (fig. 2). With the osmometer in the water bath, the meniscus in the capillary tube is observed through the micrometer after application of a pressure of 50 cm water for 10 min., during which temperature equilibrium is established. According to Holm-Jensen, the minute number M is calculated from the equation $M = \frac{1000}{V_d}$, where the micrometer is provided with 100 divisions, each division corresponding to a distance of exactly 0.005 cm in the object viewed, and where V_d is the velocity of the meniscus expressed in divisions/min. A required condition is that the ratio of the radius of the membrane to the radius of the capillary tube is 10.

Observations regarding the Permeability of the Membranes

The most suitable membrane is one that has the greatest possible permeability yet does not permit the passage of the colloids under examination. The different environmental conditions prevailing in various laboratories require that the lowest minute number at

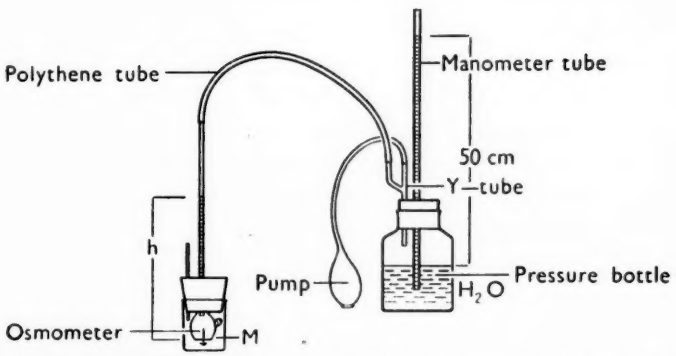

Fig. 2. — Osmometer connected to pressure bottle. — The water is filtered through the membrane (M) with pressure 50 + h—1.5 cm (1.5 capillary correction).

which the membranes are impermeable to the experimental colloids is determined before measurements of colloid osmotic pressure are undertaken.

In this investigation it was found that membranes having minute numbers of 70 and above were serviceable for the examination of serum or Macrodex. In the case of, for instance, an osmometer with a membrane whose minute number has been determined at 60, the permeability to proteins is tested as follows (Holm-Jensen 1949). The osmometer is filled with serum and connected to the pressure bottle and a pressure of 60 cm of water is applied for *c.* 2 hrs. A small amount of filtrate is carefully collected from outside the membrane by means of a capillary pipette. A drop of Spiegler's reagent (mercuric bichloride, 4 g; tartaric acid, 2 g; glycerol, 10 g; distilled water, 100 g) is placed on a micro slide and a drop of the filtrate is added. Definite precipitation of protein, which is clearly visible especially against a dark background, occurs in serum dilutions up to, at least, 1: 500, equal to a protein concentration of c. 0.01 per cent. The procedure is similar for the demonstration of Macrodex in the ultrafiltrate, with the exception that the sample is heated with hydrochloric acid (1—*N*) for 5 min. and neutralized with sodium hydroxide, and the glucose demonstrated with, for instance, Nylander's reagent, which will give a positive result at a concentration of 0.1 per cent.

Excessive density of the membrane may not only retard the passage of salts and water but also tend to give excessively high

colloid osmotic pressure values by holding back compounds that »normally» are diffused through the membrane (Krogh 1922). The blood, for instance, contains all particle sizes from crystalloids to actual colloids. For this reason, measurements were carried out with membranes having various minute numbers. Similar results were obtained with membranes gauged from 70 to 250 (table 4). With the Holm-Jensen method, equilibrium was established within 1 hr. also when membranes having a minute number of 225 were used.

TABLE 4

COLLOID OSMOTIC PRESSURE DETERMINATIONS WITH MEMBRANES OF VARIOUS PERMEABILITIES. LOW VALUES OBTAINED WITH MINUTE NUMBERS BELOW 70. (HOLM-JENSEN METHOD)

Membrane Minute No.	C.O.P. cm H_2O	Membrane Minute No.	C.O.P. cm H_2O	Membrane Minute No.	C.O.P. cm H_2O	Membrane Minute No.	C.O.P. cm H_2O
50	60.5	65	27	80	22.5	150	45
70	63	80	29	105	23.5	200	45
50	28	70	29.5	85	9	150	25
100	30.5	75	30.5	180	10	210	24.5
50	35	70	39	85	36	160	37.5
90	45	75	37	180	35.5	165	35
180	45	70	29.5	90	26.5	190	36
55	35	70	30	180	25.5	250	35
90	45	90	29	95	22.5	190	29
180	45	90	28	160	22	210	30
60	32	70	72	110	15	190	31.5
70	36	190	72	170	15	210	31.5
60	30	70	22.5	120	20	210	30
85	33	85	23.5	180	20	250	30
60	26	190	23.5	140	38	230	29
60	29	70	22	160	38	250	29
70	30	125	20	225	38		
70	31	190	20				
70	32						
70	32						

When in excess of 260, higher values began to appear and equilibrium was not obtained in 1 hr. (table 5).

Changes in temperature had no practical significance as a factor altering the properties of the membranes, even though an elevation in the temperature appeared to increase the permeability, as shown in table 6.

It frequently may be possible to carry out measurements with the same membrane for several months (table 7). The membranes were usually stored in distilled water only. In the case of infection with micro-organisms, which is shown by a change from water-clear to a dull grayish and usually also by lowered permeability, 1 drop of, for instance, BTC solution (alkyl dimethyl benzyl ammonium chloride) in concentration 1: 10,000 may be added to 5 cc of the storage water.

TABLE 5

COLLOID OSMOTIC PRESSURE DETERMINATIONS WITH MEMBRANES OF VARIOUS PERMEABILITIES. VALUES HIGHER AND EQUILIBRIUM ESTABLISHED LESS RAPIDLY WITH MINUTE NUMBERS OVER 250, (HOLM-JENSEN METHOD)

Membrane Minute No.	Colloid Osmotic Pressure, cm H_2O		
	1 hr.	2 hrs.	3 hrs.
250	34	30	
150	32	30	
260	54	40	34
120	34	31	30
260	30	28.5	26
200	26	26	27
260	28	27	27
85	24	24	24
270	37	34	34
100	30	30	29
300	60	61	61.5
185	46	45	45.5

TABLE 6

INFLUENCE OF TEMPERATURE ON THE PERMEABILITY OF MEMBRANES: CHANGES IN MINUTE NUMBERS

+20 °C	+25 °C	+20 °C	
40	30	35	
65	50	60	
70	60	68	
80	60	65	
+20 °C	+30 °C	+25 °C	+20 °C
40	25	30	35
62	48	52	70
185	125	160	185
195	135	165	185

TABLE 7

STABILITY OF MEMBRANES: CHANGES IN MINUTE NUMBERS

	June 19, 1952 →	July 21, 1952
1.	50	75
	70	90
	90	75
	180	160
	Jan. 28, 1953 →	June 6, 1953
2.	160	120
	165	200
	190	180
	200	120
	250	250

The minute numbers of the membranes were usually tested once a week, or before each measurement if a change of color had occurred. This procedure was less time-consuming than the preparation of a new membrane.

STANDARD COLLOID

The colloid used for comparison was the dextran Macrodex (Pharmacia Ltd., Uppsala, Sweden). This is a substitute for blood plasma, to be used as 6 per cent and 10 per cent infusions. The Macrodex solutions employed in this investigation were prepared from dry powder supplied by the manufacturers. The colloid osmotic pressure was determined for various concentrations of these solutions (fig. 3).

Macrodex is a polysaccharide, which is converted to glucose by acid hydrolysis. Its structure, properties and production have been described by, *e.g.*, Ingelman and Siegbahn (1944), Ingelman (1947, 1949, 1951), Ingelman and Halling (1949), Grönvall (1951) and Wallenius (1954). Dextran was discovered about the middle of the 19th century, when it was observed that this polysaccharide is formed in sugar beet juices which are infected with the bacterium *Leuconostoc mesenterioides* or similar micro-organisms. Infections by this bacterium in sugar mills had earlier caused sugar losses and slow filtration of juices containing dextran. During the present century dextran has acquired considerable importance as a substitute for blood plasma in clinical use. It is produced in biological synthesis, when enzymes of the above mentioned bacteria convert the glucose in saccharose solution to dextran. Polymerization may also be produced with cell-free enzyme solutions. Dextran differs from starch in that glucose phosphates are not present as intermediary forms during its formation and that the glucose molecule linkages are different. The dextran molecules are branched and threadlike and have a molecular weight of several million. This so-called crude dextran is subjected to acid hydrolysis, which is interrupted at a given phase, resulting in molecular sizes corresponding approximately to plasma proteins. In order to obtain such products it is possible to vary three factors: temperature, time and acid concentration. After the hydrolyzing process, the dextran may be precipitated with alcohol and dried.

Like colloids in general, Macrodex is a polymolecular substance, *i.e.*, it consists of molecules of the same chemical composition but with different molecular weights. It therefore is not to be expected that the colloid osmotic pressure of the Macrodex solutions which are produced for use as infusions would be sufficiently constant to serve as comparison in this respect. According to the manufacturer's statement, *c.* 30 per cent of the molecules are of sizes with a molecular weight below 45,000 and *c.* 25 per cent weigh over 150,000 (Ingelman 1951).

Fractionation experiments have been carried out in order to study the possibility of obtaining dextran preparations which would be more homogeneous in respect to the size of the molecules. Dextran preparations of different degrees of degradation can be divided into a number of more homogeneous fractions by means of ethyl alcohol. In this manner it is

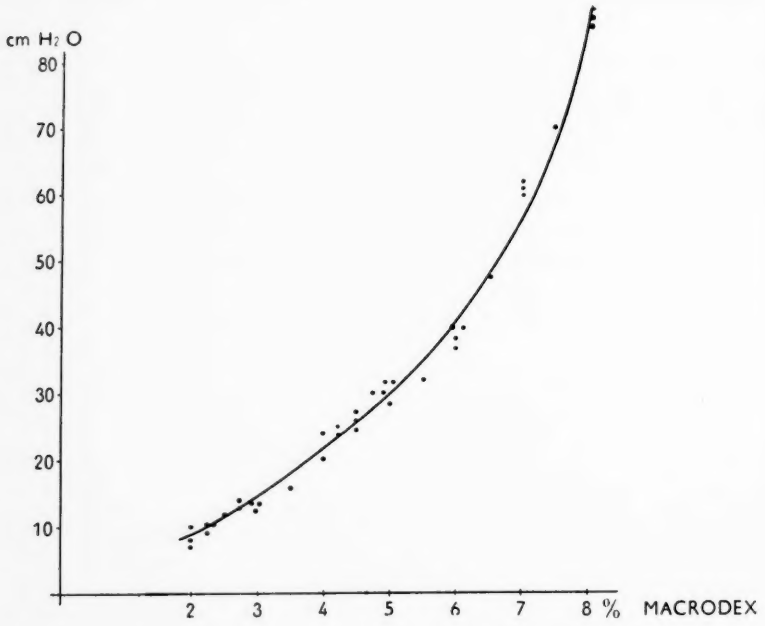

Fig. 3. — Colloid osmotic pressure of Macrodex. (Holm-Jensen method).

possible to obtain, for laboratory investigation purposes, preparations of nearly any desired molecular size which are quite homogeneous as to molecular weight (Ingelman 1951, 1954).

Macrodex is very suitable for use as a comparative solution since it remains absolutely stable in daylight and at room temperature for at least 12—18 months. The solutions can be heated without impairment to 120 °C for 30 min. The relative viscosity is slightly higher than that of plasma. The pH is over 4.5, with a low neutralization value. The freezing point lies around 0.6 °C. The nitrogen content is under 0.01 mg per cc. One gram of dextran is calculated to bind 23 cc of water. Macrodex is chemically indifferent and electrically neutral (Thorsén 1949).

DETERMINATION OF THE COLLOID OSMOTIC PRESSURE

A choice of two methods is offered. In *method I* the osmometer is filled with the colloidal solution under examination, for instance a serum, and the colloidal solution of a known colloid osmotic pressure (Macrodex) is placed outside the membrane. In *method II* the known colloidal solution is placed inside the osmometer and the solution under examination is placed outside the membrane. Both methods

will give the same result. When 1 cc or more of the solution under examination is available, method I is to be recommended, for it is more rapid and requires a smaller number of osmometers (1—2), which means that a smaller number of membranes of a suitable minute number need be used at the same time. Method II should be selected if there is less than 1 cc of experimental solution, and especially if there is less than 0.5 cc and it is doubtful whether the amount will be sufficient to fill one osmometer.

Method I

If the test solution is serum, the osmometer is washed four times with physiological saline solution drawn in through the side tube by applying suction at the end of the capillary tube. The osmometer is then washed once with the serum under examination, which is pipetted through the side tube into the osmometer chamber. The osmometer is rotated and inverted so that all parts of the chamber are moistened, and the serum is removed through the capillary tube by suction. — If a large amount of serum is available, the osmometer may be filled with serum through the side tube by suction applied at the end of the capillary tube. In this case it is necessary to rinse the outside of the osmometer very carefully after filling. Filling is preferably performed with a capillary pipette, which may be made, for instance, from a glass tube 0.5 cm in diameter by drawing it to a fine point 3—5 cm long. When the chamber is nearly full, the osmometer is tilted sideways to allow the serum to flow into the capillary tube. If it fails to flow in readily, suction is carefully applied at the end of the tube, and, if necessary, serum is added to the chamber with a pipette. Air bubbles possibly present in the chamber or the capillary tube are removed by tilting the osmometer, for no air bubbles are permissible in the tube or in the chamber at the mouth of the tube during measurement. If foam or bubbles still remain in the osmometer, they are readily removed by touching them with a horsehair dipped in octyl alcohol. After filling and before measurement is started, the surface of the fluid in the capillary tube is always to be touched with a moistened horsehair to lower the surface tension. An excessive amount of alcohol may enter the liquid in this connection, as manifested, for instance, by precipitation of proteins and turbidity on and near the surface of the

liquid. — When the osmometer has been filled and the capillary meniscus is at a height of 15—19 cm, the side tube is closed with a tight rubber cap. The outside of the osmometer (including the membrane) is rinsed carefully with physiological saline and dried.

Various concentrations of the standard colloidal solution are required. For the present experiments a 10 per cent stock solution was prepared from dry Macrodex substance dissolved in physiological saline; necessary dilutions could be prepared readily as required. For measurement of sera, 2.00—2.25—2.50—5.00 per cent solutions are used. The colloid osmotic pressures corresponding to the different concentrations are obtained from the curve in fig. 3. For the outer fluid a Macrodex dilution is selected which is considered to have a colloid osmotic effect as similar as possible to that of the serum under examination. One or two drops of this dilution are pipetted on the dry membrane of an osmometer filled with the experimental serum and on the bottom of the clean, dry »moist chamber». The osmometer is inserted into the »moist chamber» with the aid of a well-fitting rubber stopper through which the capillary tube passes. The osmometer is pressed down to a depth at which the drop of Macrodex solution hanging from the membrane touches the drop lying on the bottom of the chamber, so that the Macrodex will be held in place on the membrane. The osmometer is now placed on the rack in the water bath for observation. It is very important that the rubber stopper on the osmometer closes the »moist chamber» absolutely water-tight and that not a single drop seeps from the water bath into the moist chamber or at the sides of the capillary tube, pressure equalizer tube, or of the stopper. Since the volume of outer fluid is only *c.* 0.1 cc, even a minute small drop of water will dilute it so greatly as to give an erroneous result. — To save time, another osmometer may be filled in the same manner but with an outer fluid having a concentration for instance 1 per cent stronger or weaker. In this way it frequently is possible to localize the serum immediately between two known values.

The temperature of the osmometer, which has risen during the filling manipulation, falls to the level of the environmental temperature within 10 min. when the osmometer is held in a water bath at room temperature. By moving the osmometer, the 0 point on the micrometer is then positioned at the level of the capillary meniscus and the rise or fall of the latter is observed. The inverted image is

seen through the eye piece and the meniscus thus actually moves in the opposite direction. If the level of the serum in the capillary tube rises, the colloid osmotic pressure of the serum is higher than that of the Macrodex solution outside the membrane. In fact, it is considerably higher, since it has overcome not only the colloid osmotic pressure of the Macrodex but also the hydrostatic pressure related to the weight of its own column of fluid. If the colloid osmotic pressures on the two sides of the membrane are equal, the meniscus will fall because of the hydrostatic pressure.

When the direction of movement of the meniscus, which in this case we may assume to be an upward one, has been ascertained by observation for *c.* 5 min., the osmometer is removed from the rack. The outside of the chamber and membrane is washed several times with physiological saline and dried. The moist chamber is

TABLE 8

EXAMPLE OF A DETERMINATION OF THE COLLOID OSMOTIC PRESSURE OF A 5 PER CENT SOLUTION OF MACRODEX BY THE PRESENTED METHOD

5 per cent solution of Macrodex in osmometer. Fluid level at 19 cm. Capillary correction 1.5 cm.

13.30 o'cl.	Osmometer placed in thermostat with 2% Macrodex solution as outer fluid.
13.40 »	Meniscus rises.
14.00 »	» »
14.10 »	Outer fluid changed to 2.25% Macrodex solution.
14.20 »	Meniscus rises.
14.30 »	» » .
14.35 »	Outer fluid changed to 2.75% Macrodex solution.
14.45 »	Meniscus falls.
14.50 »	Outer fluid changed to 2.5% Macrodex solution.
15.00 »	Meniscus rises.
15.05 »	Outer fluid changed to 2.625% Macrodex solution.
15.15 »	Meniscus stable.
15.20 »	» »
15.30 »	» »
15.40 »	» »
15.50 »	» » .
16.00 »	» »

Thus the colloid osmotic pressure of a 5% solution of Macrodex = colloid osmotic pressure of a 2.625% solution of Macrodex, 12 cm of water pressure + 19 cm—1.5 cm = 29.5 cm of water pressure.

also washed and dried. A new drop of Macrodex solution, this time of a higher concentration, is placed on the membrane and on the bottom of the moist chamber. This procedure is continued until the direction of capillary movement is reversed and the sample under examination can be positioned between two concentrations of Macrodex which differ by 0.125—0.250 per cent, equal to *c.* 1.2—2.5 cm of water pressure (fig. 3; table 8).

When the measurements are completed, the osmometers are washed four times with distilled water, after which they are filled with distilled water and stored with the membrane lying below the surface of water in the moist chamber.

Method II

This method requires the use of 4—5 osmometers, for the inconvenient emptying and refilling of the osmometer during measurements should be avoided, especially since careful washing between the measurements would be necessary. On the other hand, larger amounts of standard colloidal solution are available than of the fluid under examination, and the osmometers may be filled without pipetting by using suction through the capillary tube, which effects a saving in time. Method II does not differ essentially from method I, the liquids on the two sides of the membrane only being interchanged. Thus the concentration of the liquid within the osmometer varies, whereas that of the outer fluid (the liquid under examination) remains unchanged. The smallest number of osmometers is required if one osmometer at a time is filled and the direction of movement of the meniscus is ascertained before the next osmometer is filled with another concentration of Macrodex. The velocity of the meniscus in the first osmometer may occasionally serve as an indication of whether the final value is near or very far. The following concentrations of Macrodex are then chosen accordingly.

ACCURACY OF THE METHOD AND SOURCES OF ERROR

When the colloidal solution under examination is inside the osmometer, the direction of flow across the membrane is reversed by altering the concentration of the outer standard solution (Macrodex) by 0.125 per cent, which corresponds to a colloid osmotic pressure of *c.* 1 cm of water pressure.

To prevent test errors, attention should be paid to the following points. When the osmometer is filled and the surface tension of the fluid in the capillary tube is lowered with a horsehair dipped in octyl alcohol, excess alcohol entering the serum may, as already mentioned, cause precipitation of the proteins near the surface of the fluid; this, in turn, hampers the free movement of the serum surface. Visible turbidity is a suspicious sign. — Particular care should be taken to avoid dilution of the colloid droplet used as outer fluid. When the osmometer has been removed from the water and its outside has been dried, it is well to examine that no drops of water are visible on the inner wall of the »moist chamber» or the outer wall of the osmometer chamber. If any are found, the possibilities of leakage around the rubber stopper should be sought for and corrected.

Sudden changes of temperature during the test are in principle serious sources of error. For this reason the osmometers are to be held in water during measurements. As stated above, the temperature of the osmometers, which has risen during filling, will reach equilibrium with the environmental temperature in 5—10 min., after which unexpected movements of the meniscus not related to osmosis are not likely to occur (figs. 4 and 5). Variations in the room temperature in the laboratory and in the water temperature in the thermostat, as well as movements caused by changes in temperature and not related to osmosis are shown in figs. 5 and 6.

If measurements are started before the osmometer has been kept for 10 min. in the thermostat, a fall in the capillary meniscus may occur as a result of cooling of the osmometer and not of osmosis.

CALCULATION OF THE COLLOID OSMOTIC PRESSURE FROM THE MEASUREMENTS

Forces acting in two directions are active within the osmometer. The osmotic pressure of the colloid in the osmometer seeks to hold the fluid above the membrane and to suck, if possible, more fluid inward. The adhesive force, or capillary force, between the fluid and the capillary wall has a similar action. The magnitude of this force in the osmometers used in these experiments was *c.* 1.5 cm of water pressure.[1] An opposite action is excerted by the weight of the fluid

[1] This value has been obtained experimentally by determining the capillary rises of serum and Macrodex.

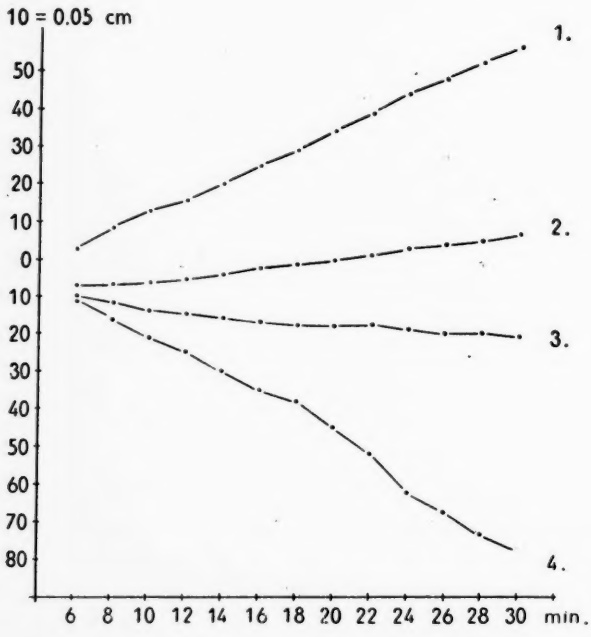

Fig. 4. — Movement of the meniscus in the osmometer capillary tube, with 10 per cent albumin solution in the osmometer. Solutions outside the membrane.

1. 3.75 % Macrodex
2. 4.00 % »
3. 4.25 % »
4. 4.50 % »

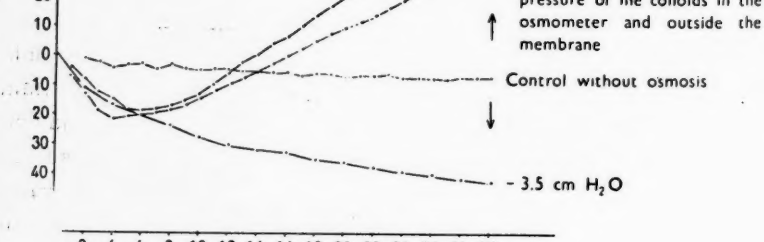

Fig. 5. — Movement of the meniscus in the osmometer capillary tube.

within the osmometer, or the hydrostatic pressure, which seeks to cause filtration of the fluid outward through the membrane. It acts in the same direction as the colloidal solution outside the membrane and its magnitude is dependent upon the height of the column of fluid, *i.e.*, on the degree to which the osmometer is filled. Reading of the fluid column is greatly facilitated if lines are drawn on the capillary tube at the levels of 10 or 15 cm.

If an unknown colloidal solution is placed in the osmometer and a known solution is outside the membrane, the osmotic pressure of the unknown solution is the colloid osmotic pressure of the known colloid + height of the meniscus in the capillary tube — 1.5 cm when the meniscus is stable. Conversely, when the osmometer contains a known colloid and an unknown solution is used as outer fluid and when the capillary meniscus is stable, the osmotic pressure of the unknown solution is the osmotic pressure of the known colloid — height of the capillary meniscus + 1.5 cm. — It is not necessary to strive to attain stability of the capillary meniscus. The object, instead, is to determine whether the colloid osmotic pressure of the liquid under investigation is higher or lower than the known pressure. *The direction of flow of the liquid indicates whether the pressure under investigation is higher or lower than the known pressure.*

Example. — The liquid to be examined is a serum, the colloid osmotic pressure of which is assumed to be 20—30 cm of water pressure. More than 1 cc of serum is available for use and method I is chosen. The osmometer is filled with the serum. Measurement shows that the height of the column of fluid is 16.5 cm. From the curve in fig. 3 it is seen that a colloid osmotic pressure of 30 cm corresponds approximately to a 5 per cent solution of Macrodex. However, this concentration is not chosen for the outer fluid, but one corresponding to a colloid osmotic pressure of 30—(16.5—1.5) cm, which is a 3.5 per cent solution of Macrodex. If the capillary meniscus begins to fall, the pressure of the serum under examination is lower than 30 cm of water pressure and a more dilute Macrodex solution is therefore placed outside the membrane. This procedure is continued until a Macrodex concentration is reached which effects a rise in the meniscus. In this way the unknown colloid osmotic pressure is confined between two known pressures.

INFLUENCE OF VARIOUS FACTORS ON THE COLLOID OSMOTIC PRESSURE

If the liquid contains several components in the colloidal state, its colloid osmotic pressure is the sum of the colloid osmotic pressures of the various components. The colloid osmotic pressure of serum is practically wholly dependent on its proteins (v. Farkas 1938, Wunderly and Wuhrmann 1947).

INFLUENCE OF LIPIDS

In addition to proteins, lipids, because of their concentration, may be capable of influencing the colloid osmotic pressure of serum. Opinions vary regarding their effect. Thus Adler (1925) observed that a colloidal solution of cholesterol had a marked capacity to bind water, whereas this was not the case with lecithin solution. According to Adler, when cholesterol is added to serum the hydrated cholesterol molecules adsorb bile acids, which in turn promote the hydration further. The opposite conclusion was drawn by v. Farkas (1926), according to whom the addition of both lecithin and cholesterol reduced the colloid osmotic pressure of serum. In his later investigations (1935) he reached the opinion that lipid emulsions play no part in this respect *in vivo*. In studying patients with hypertension, Carrière *et al.* (1936), again, observed that the colloid osmotic pressures associated with an increased total lipid content of blood were higher than would have been presumed on the basis of their protein content. In the opinion of Keys and Butt (1939), plasma lipids play no rôle in the colloid osmotic pressure. — The colloid chemical properties of blood lipids have again become topical in connection with research work on atherosclerosis.

Popjak and MacCarthy (1943) extracted from the serum the cholesterol, *c.* 2/3 of the phospholipids and *c.* 2/3 of the neutral

fats and obtained a higher colloid osmotic pressure than in the non-extracted serum. The difference, however, was small and the workers believed that some globulin had been removed at the same time, thus altering the ratio between albumin and globulin. In their opinion the lipids have no influence on the osmotic pressure. They drew the same conclusion in studying the effect of lipemia (Popják and McCarthy 1946).

OBSERVATIONS ON THE INFLUENCE OF MEALS

The author studied the colloid osmotic pressure of the sera of 6 test persons from samples drawn before the meal and 1—3½ hrs. after a high-fat meal. A marked milky turbidity of the serum 1½—2 hrs. postprandially was regarded as a sign of alimentary lipemia. As seen from table 9, no colloid osmotic pressure changes exceeding the limits of test error were seen in any of the cases. The measurements were carried out by both the Holm-Jensen method and that presented by the author.

TABLE 9

INFLUENCE OF MEAL ON THE COLLOID OSMOTIC PRESSURE OF SERUM. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE. H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

Experiment No.	Method	Before Meal	After Meal				
			1 hr.	1 ½ hrs.	2 ½ hrs.	3 hrs.	3 ½ hrs.
1	H	31.5	33	30	30	29	30
	A	30	30	30	30	30	28
2	H	30	30		29	29	30
3	H	30.5		30			
	A	28		28			
4	H	19		19	19		19
5	H	32.5		33	32		
	A	30		30	30		
6	H	38.5		38.5	38		
	A	38.5			36.5		

INFLUENCE OF ELECTROLYTES

Theoretically, electrolytes may influence the colloid osmotic pressure in at least two ways. It is a known fact that the physico-chemical state of colloids may be dependent in many ways upon the presence of electrolytes and that they also participate in Donnan's membrane equilibrium phenomenon near the membranes which are necessary for the manifestation of the colloid osmotic pressure.

The volume of a colloid is not constant even in solution and it may vary according to the ability of the hydrophilic colloid to bind more or less water. In this connection we come in contact with concepts such as »nichtlösender Raum» (v. Farkas 1938) and »fixed water», which are difficult to measure. The protein particles are usually considered to have a bipolar orientation and to be surrounded by water molecules. This »water mantle», consisting of water in a fixed, absorbed form, differs from water otherwise in the solution, for instance in respect to its capacity as solvent (v. Farkas 1938). The more »swollen» the condition of the colloid and the greater the amount of water that is bound, the less there will be free water in the solution and the greater its — so to speak — colloidal concentration and its colloid osmotic pressure. As a result, the colloid osmotic pressure of concentrated solutions may seem greater than the colloidal concentration would imply (v. Farkas 1938, Staudinger 1941) However, the real volume of the solvent must be taken into consideration in this connection. Opinions differ regarding the proportions of fixed water in the serum. According to Verney (1926), it is 50 per cent and according to Hill (1930) 10—25 per cent, with variations depending on the protein concentration, albumin/globulin ratio, and electrolytes in the solution. — Sunderman (1932), in his studies on depression of the freezing point under various conditions, concluded that the total volume of water in serum is capable of acting as solvent and thus is to be regarded as »free». — Associated with the dehydration of colloid particles is the decrease of the stability, facilitating adhesion of the particles to form aggregates. This phenomenon is seen in the serum in numerous diseases. Factors which may produce this condition are termed sensitizers; *electrolytes*, for instance, may act as such (Bladergroen 1949). A reduced colloidal stability may also bring about difficulties in measurement of the colloid osmotic pressure,

as observed by Yanagi (1935) especially with respect to pathological sera.

The rôle of Donnan's membrane equilibrium is a question which is still more closely related to the influence of electrolytes on colloid osmotic pressure. Although the membranes freely permit the passage of ions, the latter, in the presence of colloid, are unequally distributed on the two sides of the membrane. Proteins are considered ampholytic in character, *i.e.*, they may occur as both anions and cations according to the pH of the solution. Normally the serum protein has a negative charge (Nitschke 1928, Broch 1953). The charged protein acts as a colloidal electrolyte, which attracts particles oppositely charged and rejects those similarly charged (Róna and Hoffenreich 1935). As a result, the concentration of the ions charged similarly to the colloids is greater on the colloid-free side of the membrane than on the colloid side, and, conversely, the number of ions charged unlike the colloid is greater on the colloid side of the membrane than on the colloid-free side.

For this reason some proportion of the electrolytes in the solution are incapable of moving freely through the membrane and they therefore participate in the osmotic pressure produced by the colloids. Osmotic pressure, as is known, arises only from the unequal distribution of particles on the two sides of the membrane, quite independent of the nature of the particles. If permeation through the membrane is prevented for a reason other than the size of particles in relation to the »holes» of the membrane, the osmotic action still remains the same. At the isoelectric point the dissociation of proteins is at its minimum and the effect of the Donnan equilibrium is lowest, and it has been demonstrated that the colloid osmotic pressure is at the same time at its minimum (Loeb 1922b). It has been suggested that *c.* 20 per cent of the colloid osmotic pressure of the blood is a result of the unequal distribution of ions (Van Slyke 1926, Butt and Keys 1937). According to Hecht (1925), one-half of the measured colloid osmotic pressure is associated with the number of protein particles and one-half should be attributed to the Donnan equilibrium and to »fixed water». Other opinions, again, claim that the Donnan equilibrium reduces the measured colloid osmotic pressure (Wreschner 1929, Bladergroen 1949, Bull 1951).

From the practical aspect, the main point of the entire question

is the extent to which possible *changes* in the salt concentrations within physiological limits may alter these factors and thus modify the colloid osmotic pressure in either direction. Since it is difficult to measure the part played by the Donnan equilibrium or to determine the various degrees of hydration of proteins, the entire question revolves around the influence of different salt concentrations on the colloid osmotic pressure under measurement.

The serum contains over 300 milliequivalent monovalent ions per liter of serum, and proteins are capable of binding *c.* 12 milliequivalent bases per liter (Van Slyke 1926). Thus the ratio of the molar concentrations is not greatly altered by changes in the salt concentrations within physiological limits. For this reason these changes can be expected to have no significant influence on the colloid osmotic pressure of serum when the effect of, for instance, the Donnan equilibrium remains practically constant (v. Farkas 1938).

Since, however, disturbances in the amount and distribution of electrolytes in the intracellular and extracellular fluids are closely related to disturbances in the fluid balance, the influence of salts on the colloid osmotic pressure has also been a subject of investigation. It was demonstrated by v. Farkas (1926) that the colloid osmotic pressure attained its maximum in a 0.8—0.9 per cent concentration of sodium chloride and that higher or lower concentrations reduced the colloid osmotic pressure. This effect increased with increasing serum globulin concentrations. Krogh and Nakazawa, on the contrary, found no changes in the colloid osmotic pressure of serum even with when the amount of salt was doubled or cut in half. Torbert and Cheney (1936) observed reduced values in two test persons after the ingestion of sodium chloride. Kesselman (1950) reported that also isotonic saline solution has a similar effect and that, conversely, a reduced serum sodium content increases the colloid osmotic pressure. The colloid osmotic pressure was not measured by these investigators but merely calculated, and criticisms have been made concerning the results (Scathard 1951). According to Ambard and Trautmann (1945), the results differ greatly if the osmometer membrane is placed against a salt solution or distilled water. In the latter case the values may be up to twice as high, and according to these workers a hypotonic solution raises the colloid osmotic pressure of albumin, whereas globulins have a tendency to become precipitated.

There are, accordingly, two diverging points of view regarding the influence of sodium chloride on the colloid osmotic pressure. According to one opinion, sodium chloride has no effect when within physiological limits, and according to the other an increase in its concentration lowers the colloid osmotic pressure.

OBSERVATIONS ON THE INFLUENCE OF SODIUM CHLORIDE

In the present investigation the effect of sodium chloride added to serum and to different concentrations of Macrodex solution was studied *in vitro*. Comparisons were also made of the colloid osmotic pressures of various concentrations of Macrodex dissolved in saline solution and in water. Five serum samples were examined. Each sample was divided into two parts, and to one part was added 10—15 mg of sodium chloride per 1 cc of serum, thus exceeding several times the physiological concentration. Each half-sample was subdivided further into two parts and colloid osmotic pressure measurements were made by the method of Holm-Jensen and that described above by the author. The results are shown in table 10. The

TABLE 10

EFFECT ON COLLOID OSMOTIC PRESSURE OF SODIUM CHLORIDE ADDED TO SERUM SAMPLES. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE. H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

Experiment	Method	Before Addition	Added Amount		
			10 mg/cc	12.5 mg/cc	15 mg/cc
1	H	33	32		
	A	32	32		
2	H	30		29	
	A	31		30	
3	H	25			24
	A	25			25
4	H	23.5			24
	A	22			22
5	H	23	22		21
	A	25	23		25

addition of sodium chloride was not found to have altered the colloid osmotic pressure of the serum in any of the cases, although the pressure of two sera was already initially conspicuously low and the »colloid stability» might have been presumed to be below normal.

A similar result was obtained in the measurements of Macrodex solution, which are shown in table 11. Solutions of 3.5—4.0—4.5—5.0—6.0—7.0—8.0 per cent Macrodex were prepared with two solvents, *i.e.*, distilled water and physiological saline. As seen in table 11, the colloid osmotic pressures in the different concentrations did not differ by more than 2 cm of water pressure in the measurements made by the Holm-Jensen method. When measured by the author's method, a difference of 3 cm was seen in the 8 per cent concentration. However, this cannot be regarded as significant in consideration of the other results obtained.

Thus the addition of sodium chloride was found to have no influence on the colloid osmotic pressure of the Macrodex solution in these concentrations or of the sera examined. Since the colloid

TABLE 11

EFFECT ON COLLOID OSMOTIC PRESSURE OF SODIUM CHLORIDE ADDED TO MACRODEX SOLUTIONS. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE. H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

Macrodex Concentration, per cent	Method	Diluent	
		Distilled Water	Physiological Saline
3.5	H	15	16
	A	16	16
4.0	H	20	22
	A	18	19
4.5	H	27	27
	A	27	27
5.0	H	30	29
	A	28	30
6.0	H	39	37
	A	37.5	38
7.0	H	61	62.5
	A	59	59
8.0	H	86	88
	A	87	84

osmotic pressure of Macrodex proved to be the same in the presence and absence of sodium chloride, it appears probable that the Donnan effect did not occur in these measurements.

The results obtained by the two methods employed were concordant.

INFLUENCE OF THE pH

Phenomena associated with the hydrogen ion concentration influence the colloid osmotic pressure chiefly through electrolytes. The intensity of the Donnan effect is dependent on the pH of the solution in so far as the degree of dissociation and ampholyte character of the protein are dependent upon it. Furthermore, the »internal lability» inherent in proteins depends on the acidity of the solution (v. Farkas 1938, Wuhrmann and Wunderly 1947).

As is the case with salts, the question of the effect of pH on the colloid osmotic pressure concerns chiefly the effect of changes occurring within physiological limits.

Heinz and Netter (1947) have described a regulatory mechanism which maintains the colloid osmotic pressure of the blood constant and is associated with the degree of acidity of the blood. According to these investigators, the colloid osmotic pressure tends to decline as the carbon dioxide pressure increases. The fall in the pressure stops because the red cells at this time begin to take up water and to swell and the plasma protein concentration increases, thus restoring the colloid osmotic pressure (so-called Hepp effect). In exceptional cases only are the changes in the pH of the blood greater than 0.1, which corresponds to pressure fluctuations of 3—4 mm of water pressure. Thus these are changes which practically are incapable of measurement. Although in the blood circulation the changes in carbon dioxide concentration do not demonstrably alter the colloid osmotic pressure, tests with serum *in vitro*, have given evidence that a fall in osmotic pressure is associated with a reduction in pH (Heinz and Netter 1949). In studying the relationship between acidity of exudate and the osmotic pressure, Schade *et al.* (1926) observed that with a decline in the pH of the exudate a decrease also occurred in the colloid osmotic pressure. The minimum lay between the isoelectric points of serum globulins and albumin,

at pH 5.4—4.7. Any further decline in the pH was accompanied by an increase in the colloid osmotic pressure. The following values have been selected from the results obtained by Schade *et al.* (1926).

pH 8.45 = colloid osmotic pressure 1.8 cm of water pressure
pH 4.82 = » » » 0.66 » » » »
pH 3.43 = » » » 1.0 » » » »

The test error was stated to be 0.2 cm of water pressure.

Kasugai (1935), after allowing dogs to breathe low-oxygen air containing carbon dioxide, observed that as the carbon dioxide level in the blood increased, the colloid osmotic pressure declined to a greater degree than simultaneous change in proteins would have implied. A similar result, however, was obtained in the osmotic pressure when the blood carbon dioxide level declined.

In studying differences in the colloid osmotic pressure of arterial and venous blood, Kessler and Paulsen (1935) found, it is true, slightly higher values in the first mentioned but believed that the carbon dioxide content had no part in this. Butt and Keys (1937) also concluded that physiological variations in the pH cause no change in the colloid osmotic pressure. The same conclusion had been drawn earlier by v. Farkas (1926), Krogh and Nakazawa (1927) and Hirota (1928). A very slight increase in colloid osmotic pressure on the addition of OH-ions to the solution was observed by Marrack and Hewitt (1927) and Meyer (1931 c). According to Hepp (1938), the effect of carbon dioxide is not clear, but the colloid osmotic pressure rises with increasing pH values.

Thus there appear to be two differing opinions concerning the influence of pH on colloid osmotic pressure. According to one view, reduced pH values are accompanied by a lowered colloid osmotic pressure, and *vice versa*, whereas the other opinion holds that variations in pH within physiological limits have no effect on the colloid osmotic pressure.

OBSERVATIONS ON THE INFLUENCE OF THE PH

The colloid osmotic pressures of a 5 per cent solution of Macrodex and of samples of serum were examined at various pH levels, using Holm-Jensen's and the author's methods. The pH of the stock

solution of Macrodex was 6.1. The addition of 1 to 5 drops, respectively, of 0.1 per cent acetic acid to 5 cc of Macrodex gave solutions with pH 5.8—5.6—5.2—5.0—4.8. The acidity was tested with the pH meter (Radiometer, Copenhagen), which is accurate to 0.1—0.2. Similarly, the addition of drops of 1.0 per cent sodium carbonate to samples of 5 cc of Macrodex yielded solutions with pH 6.8—7.2—7.6—7.8. The measurements of colloid osmotic pressure were carried out immediately by the above mentioned methods. However, no noteworthy changes were seen in the values (table 12).

TABLE 12

INFLUENCE OF pH ON THE COLLOID OSMOTIC PRESSURE OF A 5 PER CENT SOLUTION OF MACRODEX. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE. H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

pH	4.8	5.0	5.2	5.6	5.8	6.1	6.8	7.2	7.6	7.8	
C.O.P.	29	31.5	30	31.5	31.5	31.5	31.5	30.5	32	32	H
	31	31	31	31	31	32	32	32	32	32	A

TABLE 13

INFLUENCE OF pH ON THE COLLOID OSMOTIC PRESSURE OF SERUM SAMPLES. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE. H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

1	pH	6.8	7.4	7.8			
	C.O.P.	31.5	32	32			H
		30	31	31			A
2	pH	4.8	5.0	5.8	6.6	7.4	
	C.O.P.	26 ?	26 ?	26	29	32	H
		27	28	28	30	34	A
3	pH	7.6	7.8	8.2	8.7		
	C.O.P.	34	35	34	34.5		H
		36	37	37	36		A

The effect of changes of pH on the colloid osmotic pressure of serum was studied with 12 samples of serum from 3 persons. After the determination of the initial pH of each serum, 1—4 drops of 1—2.5 per cent acetic acid or of 1 per cent sodium carbonate were added to samples of 4 cc of serum. The pH measurements were

repeated and each sample was divided into two portions, on which the colloid osmotic pressure was immediately determined by Holm-Jensen's and the author's methods. Table 13 shows the results obtained. They indicate that within the »physiological range» of pH fluctuations there were no changes in the colloid osmotic pressure that could be regarded as significant, whereas lower values were seen at levels below pH 6. The samples with a pH of 5.0 and 4.8 showed a fine protein precipitation, which rendered the colloid osmotic pressure values for these samples uncertain. A definite state of equilibrium could not be established by the Holm-Jensen method.

INFLUENCE OF THE TEMPERATURE

In theory, the temperature at measurement has a relatively slight influence on the colloid osmotic pressure. The absolute temperature T is included in the equation for osmotic pressure $p = RTC$ (in which R = gas constant and C = molar concentration). A change of 20 degrees in the temperature will change the measurement values by less than 10 per cent, or in the colloid osmotic pressure of the serum by less than 3 cm of water pressure. This lies very close to the limits of methodic error.

Several investigators have nevertheless considered it desirable to carry out colloid osmotic pressure measurements, especially that of serum, at 37 °C in order to be as close to physiological conditions as possible (Schade and Claussen 1924, Bataillard 1951). Wells *et al.* (1935) suggested that when measurements are made at 20 °C the values prevailing at body temperature will be obtained by adding 6 per cent. Krogh and Nakazawa (1927) found that temperatures between 0 °C and 37 °C had no influence on the colloid osmotic pressure of the serum. Wells *et al.* also observed that the osmotic stability of the serum is maintained for at least 24 hours at temperatures between 0 °C and 37 °C. According to them, cooling of the serum usually causes no change in the proteins, but irreversible changes may occur near 0 °C. At temperatures above 25 °C, again, putrification may readily set in. The room temperature is a practical measuring temperature. To prevent changes in the proteins in particular, Adair (1925 c) and Keys and Taylor (1935) used measuring temperatures near 0 °C.

The absolute level of the temperature during measurement therefore is not greatly significant. The important factor is maintenance of the temperature during the test without marked changes. Most of the methods for colloid osmotic pressure measurements call for the use of a thermostat, since the capillary tube of the osmometer readily acts as a »thermometer», as the inner liquid changes in volume according to the temperature. Thus the capillary meniscus may move fully independently of osmotic forces.

OBSERVATIONS ON THE INFLUENCE OF THE TEMPERATURE

The capacity of the osmometers used in the present investigation was *c.* 0.3 cc. A rise in temperature from 15 °C to 20 °C would cause a rise of *c.* 0.7 mm in the fluid level in the capillary tube due to change in volume of the fluid. In addition there would be changes in the glass. Actually, however, the meniscus makes a considerably greater movement. Changes in the temperature produce changes in the membrane, and its tension or relaxation influence essentially the volume of the osmometer.

In the presented method it was found to be especially important to obtain certainty that the movement of the fluid during the short time of observation is not a result of transitory changes im temperature. When the membranes were tested at room temperature the results were indefinite, but the situation was essentially corrected when the osmometers were placed in the water bath. Fig. 6 shows the results obtained at intervals of 1 min. during 1 hour's measurements of the laboratory room temperature and the temperature of the thermostat water, where there was no temperature control. The laboratory door was open and movement through it was not restricted in any way. A Beckmann thermometer (accuracy *c.* 0.01 °C) was used for the temperature measurements. — Fig. 5 shows the movements of the capillary meniscus during 30 min. as a result of temperature fluctuations when the osmometer is held in the thermostat and osmosis is not arranged. In Fig. 5 there is also the typical phenomenon where the meniscus begins to fall immediately after immersion of the osmometer in the water. About 6—10 min. later this movement stops and the fluid begins to move by osmotic force. The extent and duration of the initial

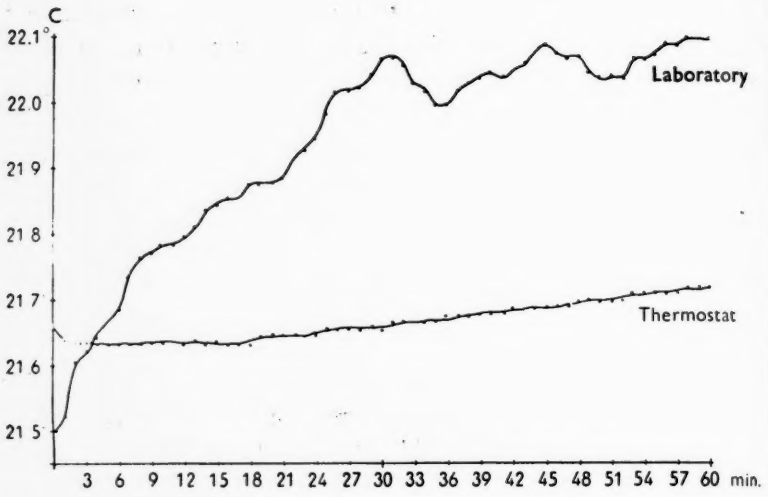

Fig. 6. — Temperature fluctuations in the laboratory and in the thermostat.

»movement by temperature» are dependent upon the difference in the temperatures of the osmometer and the thermostat water.

When the thermometer was inserted through the stopper into the »moist chamber» it indicated the temperature of the thermostat 4 min. later at a room temperature of 20 °C and a thermostat temperature of 30 °C. With the thermostat set at 35 °C the thermometer in the »moist chamber» indicated this temperature after 7 min., and after 12 min. with the thermostat at 40 °C.

The colloid osmotic pressure of Macrodex was measured at various temperatures by the methods of Holm-Jensen and the author, using solutions of 2.00—2.25—2.50—2.75—3.00—3.25—4.25—4.50—4.75—5.00 per cent. The results are shown in table 14. No changes demonstrable by the measurements were produced in the colloid osmotic pressure by the changes in temperature. The higher pressure values theoretically associated with higher temperatures differed so slightly from the values at lower temperatures that the changes come within the limits of test error. Table 14 is especially illustrative since it shows the great similarity of the results obtained with the two methods.

In view of the observations described here, the usual room temperature was considered an appropriate working temperature,

and a 30—40 liter aquarium vessel without temperature control an adequate thermostat. The measurements also seemed to indicate that equilibrium is not attained by the Holm-Jensen method within 1 hr. at a temperature of *c.* 20 °C, but that 1½—2 hrs. are required when the pressures are *c.* 30—40 cm of water pressure or higher.

TABLE 14

INFLUENCE OF TEMPERATURE ON THE COLLOID OSMOTIC PRESSURE OF MACRODEX SOLUTIONS. COLLOID OSMOTIC PRESSURE IN CENTIMETERS OF WATER PRESSURE, H = HOLM-JENSEN METHOD. A = AUTHOR'S METHOD

Macrodex Concentration per cent	Method	Temperature				
		+20 °C	+25 °C	+30 °C	+35 °C	+40 °C
2.00	H	8	8	8	9	8
	A		10	10	9	9
2.25	H	12	12	11	12	12
	A	11	11	10	10	
2.50	H	13	13	14	14	14
	A	12	13	13	13	13
2.75	H	15	17	15	15	15
	A	14	15	14	13	14
3.00	H	17	18	18		
	A	17	17	17		
3.25	H	18	19	19	20	20
	A	20	21	20	21	21
4.25	H	25	24		23	23
	A	24	24		24	24
4.50	H	26	27		24	24
	A	26	26		26	26
4.75	H	30	31			
	A	30	30			
5.00	H	32	32			
	A	31	32			

MEASUREMENTS OF THE COLLOID OSMOTIC PRESSURE OF SERUM ALBUMIN

Solutions of 5.00—6.25—7.50—8.75—10.00—15.00—20.00 per cent dry serum albumin were prepared with physiologic saline and osmotic pressure measurements were carried out by the Holm-Jensen method and the method presented by the author. The correlation of the colloid osmotic pressure to the albumin concentration is shown in fig. 7.

Calculation of the molecular weight of albumin from the osmotic pressure of a 5 per cent solution (17 cm water pressure or 0.0167 atm. pressure) will give *c.* 72,000.

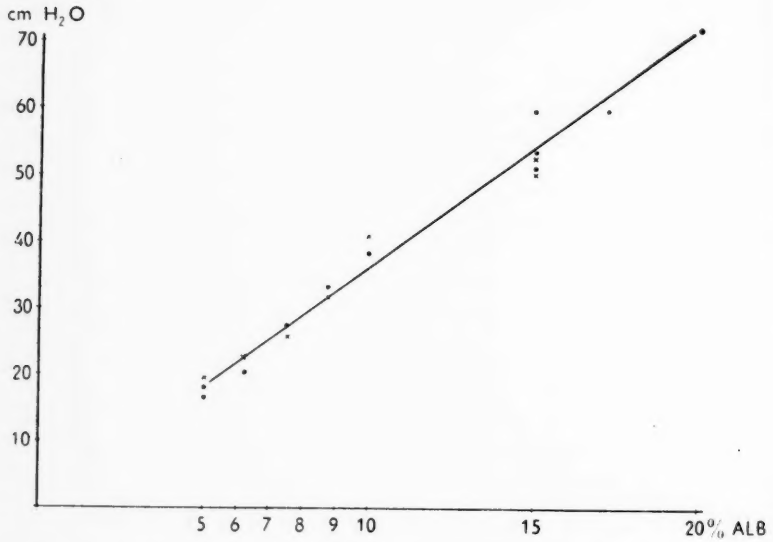

Fig. 7. — Colloid osmotic pressure of serum albumin.
• Holm-Jensen method
× Author's method

$$p = R\,T\,\frac{c}{M}$$

$$p = 0.0167 \qquad R = 0.0821 \qquad T = 273 + 20 \qquad \frac{c}{M} = \frac{50}{X}$$

When the molecular weight is calculated from the osmotic pressure of a 15 per cent solution (52 cm water pressure or 0.05 atm. pressure), then $p = 0.05$ and $\frac{c}{M} = \frac{150}{X}$. The molecular weight is c. 72,000. The results obtained correspond to the values which generally have been reported as the molecular weight of human serum albumin.

In both low and high albumin concentrations the two methods thus gave analogous results.

MEASUREMENTS OF THE COLLOID OSMOTIC PRESSURE OF SERUM

NORMAL SERA

Colloid osmotic pressure measurements were made of the sera of 20 obviously healthy persons. These were 5 female and 15 male subjects aged 20—40 yrs. The samples were taken in the forenoon and no attention was paid to meals. From 5 to 10 cc of blood was taken into a test tube from the cubital vein, using a torniquet and taking care to avoid hemolysis. After clotting the sample was centrifuged for 10—20 min. (*c.* 3 000 r.p.m.) and the serum was transferred into another tube and placed in the refrigerator. The determinations were made within 1 hr. after taking of the sample.

The results are shown in table 15. In measurements by the Holm-Jensen method the values ranged from 26.0 to 44.5 cm and by the author's method from 28.0 to 41.5 cm of water pressure. The mean values were 34 cm and 33 cm of water pressure, respectively. Two or more determinations were made of all measurements by the Holm-Jensen method. The maximum difference between the values in the different determinations was 2 cm of water pressure, no reason for the difference being found. The results shown in the table are the mean values for the determinations. In the measurements by the author's method the direction of flow of the meniscus at the extreme values of the standard colloid were examined twice.

PATHOLOGIC SERA

The sera of 20 patients with various diseases were examined and the results are shown in table 16. An attempt was made to secure sera with different protein compositions. The total protein content

TABLE 15

COLLOID OSMOTIC PRESSURE OF NORMAL SERA, IN CENTIMETERS OF WATER PRESSURE

Serum No.	Holm-Jensen Method	Author's Method
1	26	28
2	29	30
3	29	29
4	30	28.5
5	30	29
6	31	29
7	32.5	31
8	33	32
9	33	32
10	33	32
11	33.5	31
12	34	36
13	35	33
14	35	34
15	35	34
16	35.5	35
17	40	38
18	40	41
19	42.5	40
20	44.5	41.5

varied from 2.7 to 11.2 per cent and the colloid osmotic pressure correspondingly from 12 (10) to 62 (59) cm of water pressure.

The two lowest values (1 and 2) are doubtful, as the colloid osmotic pressure continued to decline. With the exception of sample 20, which had the highest colloid osmotic pressure and a difference of 3 cm of water pressure between the two methods, the results for the measurements by the two methods were uniform and the differences were of the order of 2 cm of water pressure.

TABLE 16

PERCENTAGE OF TOTAL PROTEIN AND COLLOID OSMOTIC PRESSURE OF SERUM IN CENTIMETERS OF WATER PRESSURE IN VARIOUS DISEASES

Serum No.	Diagnosis	Holm-Jensen Method	Author's Method	Total Protein %
1	Nephrosis	12	10	2.7
2	Chr. nephritis	15	12	4.1
3	Congestive heart failure	19	18	4.8
4	» » »	20	20	5.0
5	» » »	21	20	5.2
6	Lymphogranulomatosis	22	20	4.4
7	Congestive heart failure	26	24	5.7
8	Exudative pleurisy	26	28	
9	Coronary infarction	29	30	
10	Rheumatic fever	30	30	5.5
11	Congestive heart failure	32	29	6.0
12	» » »	32	34	
13	Myelomatosis	37	34	9.6
14	Hyperglobulinemia	38	37	8.9
15	»	40	42	8.9
16	Malaria	41.5	43	10.2
17	Myelomatosis	42	42	8.2
18	»	44	47	10.2
19	»	45.5	45	11.0
20	»	62	59	11.2

DISCUSSION OF THE RESULTS

It appeared from the chapter describing various methods of colloid osmotic pressure measurements that a number of reliable techniques are available which are based on the compensation of osmotic pressure. The drawbacks common for all of these methods were found to be the length of time required for the measurement and the complicated equipment typical of the more rapid micro-methods, clinical use in particular being limited by these factors.

In the method presented in this report an attempt was made to minimize these two drawbacks. Its principle is more close to the conditions in nature than that of the usual methods of measurement based on the static and compensation principles. In biological systems, substances in the colloid state are present on *both* sides of semipermeable membranes or interfaces. Thus there generally exist no colloid-free »external» salt solutions, which are characteristic of osmometers.

The direction of flow of the fluid, on the observation of which the method is based, is dependent on the difference in the concentrations of the fluids on the two sides of the membrane when the hydrostatic pressure and capillary force have been taken into consideration. The difference gradually levels off, and the flow diminishes and discontinues entirely when the state of equilibrium is attained. However, the movement continues for several dozens of minutes in the presence of the volumes of fluid and differences in concentration used in this investigation. — Since it is necessary to change the concentration of the colloid on one side of the membrane a number of times, it must be possible to determine the direction of flow accurately and rapidly in order to save time. As was seen in figs. 4 and 5, it may be determined in 6—8 min.

In the osmometers used in the present investigation the ratio of the diameters of the capillary and the membrane was 1: 10. Using a microscope, it is possible to clearly discern movements of 0.1 mm

in the column of fluid. Accordingly, a rise of 0.1 mm in the capillary meniscus corresponds to the passage of an 0.001 mm thick fluid layer through the membrane into the osmometer. Thus it is possible to determine the movements of a fluid volume which is very minute in comparison to the volume of fluid within the osmometer. When it has been ascertained that this slight flow of fluid is not produced by changes in the temperature, action of electrolytes, or external factors related to the osmometer, but is due to actual »colloid osmosis», a theoretic basis may probably also be found for this principle of measurement involving »sample tests». The present work has not been concerned with this aspect of the matter.

The Holm-Jensen method, which was used as a comparison of the presented new method, was employed in this connection for over 200 determinations of colloid osmotic pressure. Measurements of sera, for instance, gave values of the same magnitude as the normal values reported in the literature (Meyer 1932, v. Farkas 1938). The methodic error associated with the method was also of the magnitude common in these measurements. Thus the Holm-Jensen method may be considered suitable for use as a comparative method.

In an attempt to find the smallest difference in concentration which is capable of changing the direction of flow across the membrane and in the capillary tube, the answer was obtained by direct observation of the osmometer. As seen in table 8, it was possible to stop the rise of fluid in the capillary by substituting outside the membrane a concentration of 2.625 per cent Macrodex for the 2.50 per cent concentration. As shown in the curve in fig. 3, this difference in concentration is equal to a difference of *c.* 0.5 cm of water pressure in the colloid osmotic pressure. — The reliability of the method, again, was tested by the measurement of different colloidal solutions under variable conditions and by comparison of the results with those obtained by the Holm-Jensen method. In all the measurements an effort was thus made to determine, on the one hand, the colloid osmotic pressure in each case and, on the other hand, the divergence of the values obtained by the two methods.

The colloid osmotic pressure measurements of serum albumin indicated that both methods yielded results which were identical in so far that the maximum deviation was 2.5 cm of water pressure. Calculation of the molecular weight of albumin from the results gave a weight of c. 72,000, which corresponds to the weights

obtained by Burk and Greenberg (1928) and Adair and Robinson (1930). Wuhrmann and Wunderly (1947), on the other hand, reported a molecular weight of *c.* 60,000.

The largest number of parallel measurements were made with various concentrations of Macrodex. The colloid osmotic pressures corresponding to the different Macrodex concentrations were seen in fig. 3. This curve was drawn on the basis of measurements by the Holm-Jensen method, and it was always used in the calculation of the colloid osmotic pressure from the results of measurements by the technique presented by the author.

A total of 63 parallel determinations were carried out with various concentrations of Macrodex, using the two methods, and the results were shown in table 8, 11, 12 and 14. It will be observed that the results diverged only by 1—2 cm of water pressure.

Macrodex has proved serviceable for use as the comparative colloid in the presented method. The 10 per cent stock solution from which the dilutions were prepared was found to be highly stable, for dilutions made 12 months after preparation of the stock solution had the same colloid osmotic pressures as dilutions made from a fresh stock solution. The bottle was stored at room temperature and in daylight. Thorsén (1947), using other physical measurements, found that the Macrodex was absolutely stable for 12—18 months and that the dilutions may be heated to 120° C for 30 min. without damage. The possibility of heating the solution is an advantage not only from the point of sterilization but also for the preparation of hypertonic standard solutions from the dry substance, as Macrodex is readily soluble in hot water.

On examining the results in tables 15 and 16 it is observed that the two methods yielded practically similar results for 20 normal sera and 20 pathologic sera. Although the results diverged more than those for Macrodex, the maximum deviation did not exceed 3 cm of water pressure. Reduced colloid stability of the serum may probably be one of the reasons for the diverging results, specially for the pathologic sera. Sudden changes may occur in the colloid osmotic pressure, particularly in pathologic sera with a low protein content (Yanagi 1935). It was not always possible to carry out both measurements simultaneously and in some cases there was an interval of several hours.

The colloid osmotic pressures of the normal sera are well in

agreement with the normal values reported in the literature. A mean value of 34 cm of water pressure was obtained by the Holm-Jensen method, and 33 cm by the author's method. The normal pressures reported in most investigations lie between 33 and 36 cm of water pressure (v. Farkas 1938).

The observations presented above thus appear to indicate that the method here presented is serviceable for measurement of the colloid osmotic pressure of albumin and dextran solutions and of sera. Within the limits of methodic error, the results generally were of the magnitude obtained with the Holm-Jensen method.

In addition to the parallel control of the methods, an attempt was made to study the possible influence of lipids, sodium chloride content, pH and temperature on the results of the measurements by the methods in question.

Since diverging opinions have been presented on the effect of serum lipids on the colloid osmotic pressure, it was considered desirable to study whether a fatty meal has any effect on the results of measurements. Sera were selected which showed a milky cloudiness after such a meal. It was seen from table 9 that the fat content of the serum seemed to have no effect on the results and it thus appears that the colloid osmotic pressure is not influenced by the food intake in at least this respect. A similar conclusion was earlier drawn by, *e.g.*, v. Farkas (1935), Keys and Butt (1939) and Popják and McCarthy (1943 and 1946).

Fahr and Swanson (1926) and Kylin (1931) have observed that also a high-protein diet is unable to elevate the colloid osmotic pressure of the blood.

In studying the possible influence of pH on the colloid osmotic pressure, it was observed that in the range of pH 4.8—7.8 the colloid osmotic pressure of a 5 per cent solution of Macrodex remained unchanged. In serum, also, no essential differences were observable in the pressure within the range of possible physiological variations. The reduction in pressure on shift toward the acid side was shown in table 13. From the point of colloid osmotic pressure measurements it may thus be regarded as inconsequential whether or not the persons under examination were on an acidifying or an alkalinizing diet. Earlier investigations showed slight or no changes. Thus Blum *et al.* (1928) reported a slight reduction in the colloid osmotic pressure from ingestion of ammonium chloride or sodium bicarbon-

ate. Meyer and Friedheim (1931), after administration on several days of large doses of ammonium chloride to test persons,observed decreasing colloid osmotic pressure values as the alkali reserve diminished, whereas sodium bicarbonate gave variable results.

From table 13 it was seen not only that the colloid osmotic pressure of the serum declines on approaching the iso-electric points of albumin and globulins, but also that the values obtained by the Holm-Jensen method were lower and that the state of equilibrium was not attained in two cases. This may possibly be ascribed to denaturation and incipient precipitation of protein due to added acid, which is more clearly manifested in the Holm-Jensen method because of the longer time required for measurements.

A low-sodium diet ingested over a long period has been found to be capable of producing a slight rise in the colloid osmotic pressure, and a sodium-rich diet a reduction (Del Baere 1931, Meyer 1931 b, Kesselman 1950). However, the changes were so slight as to probably be of no practical significance from the point of the presented method.

The influence of sodium chloride was studied by adding it to the serum samples and Macrodex solutions, with the results shown in tables 10 and 11. Since all the measurements indicated that sodium chloride causes no demonstrable change in the colloid osmotic pressure measured by these methods, quantitative studies were not considered necessary. The results of the serum measurements were in agreement with those of Krogh and Nakazawa (1927).

As reported by Thorsén (1949), dextran molecules are not electrically charged, and Macrodex thus differs from most biocolloids, which are colloidal electrolytes. The results in table 11 are compatible with this, as they show that the colloid osmotic pressures were of the same magnitude regardless of whether the Macrodex was diluted in saline solution or water. Should electrical potentials be present in the dextran molecules, the presence of electrolytes would cause an unequal distribution of ions on the two sides of the membrane and the development of Donnan equilibrium. Consequently it would be expected that the measured colloid osmotic pressure would not be the same as in a salt-free environment. For this reason Macrodex may offer interesting possibilities for an investigation of the effects of membrane equilibrium.

It may appear surprising that marked differences in salt concentration did not have a disturbing effect on measurements.

It seems probable that the initial direction of flow of the fluid depends on the difference in the electrolyte concentration on the two sides of the membrane, especially in view of differences in the rate of permeation of different ions through the collodion membrane (Hecht 1925, Michaelis and Fujita 1925, Nitschke 1928). In the present measurements, however, it was the effort to use, in general, initial electrolyte concentrations which were as similar as possible on both sides of the membrane. — As was mentioned previously in this report (cf. page 27), Hansen (1952)presented in connection with his own osmometer the ideas of rapid permeation of electrolytes and *state of equilibrium established within a few minutes in very thin layers of fluid just adjacent to the membrane.* The permeability of the membranes to electrolytes is therefore presumably great, and *the greater part of the fluids both within the osmometer and outside the membrane are in a fully »passive state» during measurement.* It therefore is not advisable to stir the fluid during measurement.

In studying the effect of temperature and its changes on the results of measurements it was observed that room temperature is suitable and that a simple aquarium vessel without temperature control is adequate to keep the temperature fluctuations down to a minimum. Changes in temperature during measurement were found to be below 0.1° C (fig. 6). Actually a single measurement requires only about ten minutes to perform, during which the change in the temperature of the water is only *c.* 0.025 ° C and that of the room temperature, according to a performed measurement, *c.* 0.1° C. Although changes in the temperature of a protected room may in themselves not have a disturbing effect, immersion of the osmometer in water nevertheless proved necessary. On manipulation the osmometer becomes warm, and it cools slowly in the air but in 6—8 min. in water, as was seen for instance in fig. 5. If the osmotic changes can be equalized before the difference in temperature between the osmometer and the environment reaches equilibrium, it is obvious that no idea can be obtained of the flow of fluid in the capillary tube. For this reason, also, the osmometer should be got to the environmental temperature as rapidly as possible.

The use of a water bath adds to the inconvenience of manipulation in so far that the water-tightness of the osmometer must be watched constantly. To facilitate the changing of the outer fluid the rubber stopper may be lubricated with vaseline, so that the

osmometer can easily be pushed tightly in place, but precaution should be taken not to allow any vaseline to get on the membrane.

Wells (1932) has presented the requirements that should be placed on a good osmometer and reliable colloid osmotic pressure measurements. In his opinion, an imperfect test technique is an important reason for the diverging results obtained by different investigators. Although the criteria of Wells are intended to be applied to methods based on the principle of compensation, they may also be used for the evaluation of the method presented by the present author.

According to Wells, the membranes shall not be so tight that establishment of the state of equilibrium necessary for the determination will require more than 24 hours. Conversely, the membranes shall not be so permeable as to permit the passage of some of the colloid under examination. The specific permeability of the membranes used in the measurement, *i.e.*, the lowest minute number at which the colloid under investigation is kept back by the membrane, must be stated. The sensitivity of the method employed for analysis of the ultrafiltrate is also to be mentioned. The membrane must not be infected but must be water clear. Antiseptic substances possibly used for preservation of the membrane must not change the permeability and they must be carefully washed off before measurements are started in order to prevent precipitation of the proteins.

In the opinion of Wells, room temperature is best suited for the measurements. It is necessary that the thermostat keeps the temperature constant with an accuracy of at least 0.1° C. The volume of outer fluid should be as small as possible in order to minimize the change in the composition of the inner fluid by the diffusion phenomena. To prevent concentration of the solution under investigation, ultrafiltration at excess pressure should be avoided as far as possible. The state of equilibrium must be constant for at least two hours and differences in the results of parallel determinations must not be greater than the capillary correction.

In the method presented here the colloidal solution is kept in the osmometer for a few minutes only, instead of several hours, thus reducing to a minimum the denaturation of the colloids as a result of spontaneous changes. The effect of the dense membrane as a factor prolonging the time of measurement is not as harmful in the

present method as in methods based on the principle of compensation. The direction of flow of the fluid is in all cases the same and it is not necessary to wait a long time for the establishment of the state of equilibrium. This has the advantage that membranes may be used which are not very near the »ideal permeability», which are just capable of holding back the experimental colloids but have the greatest possible permeability to water and electrolytes, so that some of the experimental colloid might pass to the wrong side of the membrane.

As is seen in table 4, membranes having minute numbers ranging from 70 to 250 gave similar results in measurements of sera and Macrodex solutions. When the membranes are prepared in the manner described, they usually have minute numbers within this range.

The passage of the experimental colloid through the membrane to the wrong side is not liable to occur in measurements by this technique, as no excess pressure is used and the solution is kept in the osmometer for such a short time that the colloids presumably would have not had time to become diffused through, even if the membrane would permit it. In random tests for proteins after the measurements, protein was not found in the Macrodex droplet outside the membrane in a single case measured by the author's method. In measurements by the Holm-Jensen method some results had to be discarded as the membranes sometimes began to let proteins through. Controls were made of this possibility when the colloid osmotic pressure showed a continuous tendency to fall.

Since in the presented method the solution under examination, *i.e.*, the serum, is usually inside the osmometer, controls of the permeability to colloids consist chiefly of the determination of protein in the droplet below the membrane. This is demonstrable from a concentration of 0.01 per cent with Spiegler's reagent.

A change of the water-clear lucidity of the membrane to a grayish turbidity is a sign of infection. In the author's opinion even a cloudy membrane is usable provided a control of the minute number is made daily before measurements are started. Such a membrane may retain its serviceability for weeks, and even a daily control of the permeability is a simpler procedure than the preparation of a new membrane. Microbes which may have infected the membrane will not be able to cause demonstrable changes in the

colloids during the short time required for the measurement. Wells was of the opinion that the danger of infection of the colloid is slight if the determinations require less than 24 hours, whereas in the opinion of Fellows (1932) it is desirable that the work is carried out under aseptic conditions. The present writer feels that in his method the infection of the colloid to be placed in the osmometer is not a danger. On the other hand, of course, samples which possibly are prepared for later control measurements should be protected from infection and the sera kept in the refrigerator.

Although a state of osmotic equilibrium is not sought in the method presented, it can be attained and maintained (table 8). To maintain equilibrium for several hours the osmometer should naturally be kept in a controlled thermostat. Thus the osmotic pressures of two colloids, the hydrostatic pressure and the capillarity compensate each other.

The author's method calls for a small amount of outer fluid, as proposed by Wells. Usually a drop of colloid pipetted on the membrane is equal to 0.1 cc, but even a smaller droplet will spread over the surface of the membrane by being pressed between the membrane and the bottom of the moist chamber. The surface tension below the membrane, which counteracts the capillarity of the osmometer, is dependent upon the distance between the membrane and the bottom of the moist chamber. However, according to tests made, variations of a few millimeters have no practical effect. An advantage of the small amount of fluid which touches the bottom of the glass chamber instead of hanging from the membrane may also be considered to be the more rapid equilibrium of temperature than when a layer of air intervenes.

It may seem surprising that the presented method has given colloid osmotic pressure values which are similar to those obtained by compensation measurements. In other words, the colloid — Macrodex — which replaces the saline solution outside the membrane, did not alter the unequal distribution of ions which is a result of the protein on the other side of the membrane and which is considered significant as a contributor to the colloid osmotic pressure of proteins (Atchley *et al.* 1923, Govaerts 1924 b, Rusznyák 1924, Adair 1925 a, b, 1926, 1928 and 1929, Hecht 1925, Butt and Keys 1937). The previously mentioned electroneutral character of Macrodex may provide an explanation for this.

In comparison to other methods of colloid osmotic pressure measurements, the principal advantages of the method presented here are probably the *simple equipment and the relatively short time needed for measurement.* The substitution of an aquarium vessel for a controlled thermostat and the omission of Holm-Jensen's electromagnetic stirrers and their additional appliances greatly simplify the equipment. Although several successive measurements must be made in the present method, it may be regarded as a time-saving one, for the measurements were usually carried out in 30—40 minutes. It is true that the methods presented by Hansen (1952) and Pappenheimer and Lin (1953) are considerably more rapid and give the reading within a few minutes. However, the equipment required in the latter methods, including the complicated osmometers and electromanometers, are at least in their present form rather intricate.

The performance of several successive »sample measurements» adds to the dependability of the method, for the values obtained control in a way each other and erroneous readings are easily disclosed. The amount of experimental solution is nevertheless no greater than that required in other micromethods, 0.5 cc being sufficient.

One of the weak points in the present method is the problem connected with all the present osmometers — the membrane. However, it probably is not a surmountable one, even with a view to clinical application of the method, for even though the preparation of the membrane requires some experience it usually succeeds so well that the number of unserviceable membranes is insignificant. Since every membrane must be controlled, it is necessary to have a manometer system, which otherwise would not be required.

Another obvious drawback in this method is the difficulty connected with the standard colloid. Heretofore, at least, it has not seemed possible to obtain a solution which would have a known colloid osmotic pressure of such stability that it could be employed as the comparative solution. It also is not possible to prepare from dry Macrodex substance, by weighing, solutions that would have the desired colloid osmotic pressure. On the other hand, however, dextran fractions of a known, and even the desired, molecular size are available (Ingelman 1951 and 1954). The making of stable stock solutions is thus possible for the easy preparation of desired

dilutions. However, the colloid osmotic pressure of such dilutions must be measured before use.

It would be desirable to secure a common criterion of measurements by different types of osmometers and to obtain still more uniform results by having a standard solution of, for instance, Macrodex, for colloid osmotic pressure measurements. This would presumably provide possibilities for the application to clinical use of the principle of the method now presented, which in turn would stimulate the development of practical improvements, such as elimination of the hydrostatic pressure by placing the capillary tubes in a horizontal position.

Pathophysiological conditions which are manifested in numerous disturbances of the fluid balance could thus be studied from aspects which heretofore have received less attention because of practical difficulties in their examination. The therapeutic application of the method might be its use in the selection of suitable infusion solutions.

The following answers can now be given to the questions posed:

1. The colloid osmotic pressure can be measured by observation of the direction of flow between solutions of known and unknown colloid osmotic pressure. The construction of a simple osmometer acting on this principle is possible.

2. The results obtained with such a method correspond to the values obtained with the Holm-Jensen method for (a) albumin solutions, (b) dextran solutions, (c) normal sera, and (d) various pathologic sera.

3. The effects of the electrolyte concentration, the hydrogen ion concentration and the temperature of the solution under examination are of no practical importance within the physiological range.

4. Advantages of the presented method in comparison to earlier methods are considered chiefly to be the simple equipment required and the relatively great saving in time; its disadvantages are certain problems relating to the membranes and to the difficulty of procuring suitable standard colloids, which at the present time limit its clinical application.

SUMMARY

Based on reports in the literature, a survey is made of the methods for measurement of the colloid osmotic pressure, and theoretic and practical aspects relating to these methods are discussed.

It was demonstrated in a series of tests that the colloid osmotic pressure can be measured by observing the direction of flow of the liquid in the capillary tube of an osmometer when Macrodex solutions of different known osmotic pressures are placed outside the osmometer membrane.

The osmometer chamber, the upper part of which tapers to a capillary tube, is filled with *c.* 0.5 cc of the solution under examination. The bottom of the chamber consists of a collodion membrane through which osmosis takes place. Varying concentrations of Macrodex solution are pipetted on the outside of the membrane and the direction of flow of the fluid in the capillary tube is observed through an ocular micrometer. The combined forces of hydrostatic pressure, capillarity, and osmotic pressure of the known and unknown colloids move the fluid in the capillary tube, either raising or lowering the fluid level. Only the last of these four factors is unknown and can readily be determined by calculation. It is not necessary to use a controlled thermostat. The measurement requires 30—40 minutes and is accurate to *c.* $\pm$ 1 cm of water pressure.

The method was controlled by comparison of the results with parallel tests carried out by the Holm-Jensen (1949) method. Measurements were made with albumin solutions, dextran solutions, normal sera and various pathologic sera. The results obtained by the two methods were found to be similar. The molecular weight of the serum albumin calculated from the colloid osmotic pressures obtained in the measurements was *c.* 72,000.

The colloid osmotic pressure of Macrodex solutions was studied under various conditions and its dependence on concentration was demonstrated.

The colloid osmotic pressures of 20 normal sera varied between 26.0 and 44.5 cm of water pressure. The mean pressure when measured by the Holm-Jensen method was 34 cm of water pressure and by the method presented by the author 33 cm of water pressure. The maximum deviation between the values obtained for a single serum by the two methods was 3 cm of water pressure.

The colloid osmotic pressures of 20 pathologic sera ranged from 10 to 62 cm of water pressure. The maximum deviation between the two methods was 3 cm of water pressure.

Addition of sodium chloride was found to have no effect on the colloid osmotic pressure of the Macrodex solution or serum even when the physiological concentrations were greatly exceeded.

The hydrogen ion concentration had no influence on the colloid osmotic pressure of Macrodex, nor on that of serum within the physiologic range, whereas beyond this range the colloid osmotic pressures of sera decreased with increased acidity.

No influence by alimentary lipemia on the colloid osmotic pressure of serum was observed.

The influence of various temperature levels and permeabilities of membrane on the results of measurements were also studied.

Compared with other methods of colloid osmotic pressure measurement, the advantages of the method now presented are considered to be the simple equipment and the relatively short time required for measurements. The preparation of the membrane and the difficulty of procuring standard colloids are regarded as the chief disadvantages.

It is suggested that the method presented is based on the phenomenon of osmosis, which presumably always occurs in very thin layers of fluid in the immediate vicinity of the membrane. The greater proportion of both the experimental and comparative colloid solutions would thus remain outside the phenomenon of osmosis during the entire time of measurement.

ZUSAMMENFASSUNG

Eine Übersicht über verschiedene Messmethoden des kolloidosmotischen Druckes wird unter Heranziehung der Litteratur dargestellt. Theoretische wie praktische Fragen werden erörtert.

Es wurde festgestellt, dass der kolloidosmotische Druck durch Beobachtung der Strömungsrichtung in einer Osmometerkapillare gemessen werden kann, wenn an der Aussenseite der Osmometermembran verschiedene Macrodex-lösungen von bekanntem kolloidosmotischem Druck angebracht werden.

Die Osmometerkammer mit angeschlossenem Kapillarrohr wird mit cirka 0.5 cc der zu untersuchenden Flüssigkeit gefüllt. Der Boden der Kammer besteht aus einer Kollodiummembran, durch welche die Osmose stattfindet. An der Aussenseite der Membran werden verschieden starke Macrodex-lösungen angebracht, und die Bewegungsrichtung des Flüssigkeitsspiegels wird im Okularmikrometer verfolgt. Die Flüssigkeit im Kapillarrohr steigt oder sinkt unter Einfluss des hydrostatischen Druckes, der Kapillarkraft sowie des kolloidosmotischen Druckgefälles zwischen der bekannten und der zu untersuchenden Lösung. Aus den drei bekannten Grössen ist die vierte, d.h. der kolloidosmotische Druck der untersuchten Lösung leicht zu berechnen. Ein geeichter Thermostat ist für die Messung nicht nötig. Die Messung dauert 30—40 Minuten. Die Genauigkeit beträgt rund $\pm$ 1 cm H_2O.

Die Methode wurde durch vergleichende Messungen nach dem Verfahren von Holm-Jensen (1949) kontrolliert. Es wurden Messungen an Albumen- und Dextranlösungen, sowie an Normalserum und verschiedenen pathologischen Serumproben ausgeführt, — die Ergebnisse waren für beide Methoden übereinstimmend.

Anhand der Messungen wurde für das Molekulargewicht des Serumalbumens der Wert von 72.000 erhalten.

Der kolloidosmotische Druck von Macrodex-lösungen wurde unter verschiedenen Verhältnissen gemessen, sowie dessen Abhängigkeit von der Konzentration dargestellt.

Der kolloidosmotische Druck von 20 normalen Serumproben schwankte zwischen 26 und 44.5 cm H_2O. Der Durchschnittswert war nach der Methode von Holm-Jensen 34 cm H_2O, nach der Methode des Verfassers 33 cm H_2O. Der grösste Unterschied zwischen den Ergebnissen beider Methoden betrug 3 cm H_2O.

Der kolloidosmotische Druck von 20 pathologischen Serumproben schwankte zwischen 10 und 62 cm, wobei der grösste Unterschied zwischen den Ergebnissen beider Methoden 3 cm H_2O ausmachte.

Eine Veränderung des kolloidosmotischen Druckes durch Hinzufügen von NaCl konnte nicht festgestellt werden, obschon die physiologische Salzkonzentration überschritten wurde.

Veränderungen des pH wirkten nicht auf den kolloidosmotischen Druck der Macrodex-lösungen ein. In Bezug auf die Serumproben verblieb eine pH-veränderung innerhalb physiologischer Grenzen ebenfalls ohne Einwirkung auf den kolloidosmotischen Druck, wogegen eine Abnahme des pH über die physiologischen Grenzen hinaus eine Abnahme des kolloidosmotischen Druckes bewirkte.

Bei alimentärer Lipämie konnte keine Veränderung des kolloidosmotischen Druckes festgestellt werden.

Eine Untersuchung über den Einfluss von Membranen verschiedener Permeabilität sowie von der Temperatur auf die Ergebnisse der Messungen wurde anschliessend durchgeführt.

Der Vorzug der angegebenen Methode im Vergleich zu anderen Verfahren besteht in einer einfacheren instrumentellen Ausrüstung sowie in der schnelleren Messung. Die Herstellung der Membran, sowie die Schwierigkeit Standardlösungen zu erzeugen erwiesen sich als die hauptsächlichen Nachteile der Methode.

Die dargestellte Methode gründet sich wahrscheinlich auf osmotische Phänomene, welche möglicherweise innerhalb ganz dünner Flüssigkeitsschichten in unmittelbarer Nähe der Membran stattfinden. Der grösste Teil sowohl der zu untersuchenden wie der Standardlösung nimmt somit während der Messung nicht an dem osmotischen Geschehen Teil.

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